

Teaching technology needs more thought

British secondary schools seem to have a flair for making most things seem academic. Now there is a danger of them doing that to technology.

BRITAIN is now almost symmetrically ambivalent about technology. On the one hand, august committees of the House of Lords utter sombre warnings that, on present trends, Britain's exports of manufactured goods will be such a steadily declining proportion of the total during the coming decade (as they have been in the past decade) that, by the end of the century, the chance of staying economically afloat will be almost negligible. The government, on the other hand, rejects this simplistic advice (which is fair) on the grounds that service industries are as worthwhile as manufacturing industries, but also urges that Britain should become more conscious of the power of technology in the modern world in case it may be possible to earn an honest penny that way. And British secondary schools, constitutionally bereft of firm guidance, do their best to please those whose voices are the most plausible, not least by trying to teach technology in secondary schools, to students from age eleven upwards. An unreflective account of some of the nonsenses that result from these good intentions can be read in a booklet published last week by the Department of Education and Science, and which is the report of an enquiry into the teaching of technology in schools carried out by a group of school inspectors, *Technology and school science* (HMSO, £2.00).

The starting-point for enquiry is a statement that the "technological component" of the secondary school curriculum in Britain is likely to increase because of the general recognition "that we live in an increasingly technological society" and that "living and working in such a society" requires "some contact with, and appreciation of, technology". The proposition is an unexceptionable platitude from which nobody will dissent. During the past two decades of curriculum development in British secondary schools, it has from time to time been a powerful stimulus to change. Two decades ago, for example, there were several curriculum projects designed to introduce technology as a school subject fit to be taught alongside Latin and Greek as vehicles by which students could earn grades in school-leaving examinations (which remains the chief purpose of British secondary schools). Now, another wave of enterprise is under way, fanned by the interests of organizations as different as the Department of Trade and Industry and the Fellowship of Engineering, and aided by the complicity of the organizations that provide public examinations for British schools. Before the new wave of this movement gets out of hand, it would be as well that those concerned should ask themselves what they are about.

Tackling technology

Technology is every bit as important as its well-wishers say, both as a means by which countries (even Britain) can regain a semblance of prosperity and as a component of the education of young people. The immediate issue, that with which the school inspectors' report is concerned, is how it should be handled in secondary-school classrooms. The inspectors' report is an interesting and in many ways amusing account of how young people have tackled technical exercises in British schoolrooms. Young people have drilled holes in the lids of tin cans, using them to produce jets of steam that may make mock turbine-wheels spin, and have fitted clothes pegs with fins of various kinds so as to test the stability of objects moving through fluids. By most accounts, young people enjoy activities such as these,

which is not surprising. There is also some anecdotal evidence that little projects give young people confidence in tackling large projects, as some are required to do as part of formal and examined courses called by names such as "applied science and technology", which is again no wonder. The question that neither the school inspectors nor their sponsors stop to ask is what all this does for the education of young people, either as professional engineers or as citizens of a new world.

Foundations

Here are some principles from which to start. First, project work in schools is, generally, a valuable activity; there should be more of it, in all kinds of contexts, but projects should stretch the imagination and not consist (as they often do) of exercises in copying out what may be found in library books. (The inspectors agree on the last qualification.) This is a strictly educational argument, in support of which there is a host of shining examples. Second, however, there is no necessary connection between projects mounted at school and an adult's later understanding of the realities of modern technology. That lesson needs to be learned, for which purpose some experience of the inspectors' brave new world is needed. One easy way of providing that in schools is to enable young people to handle the bits and pieces of modern technology, which argues for helping them to tinker with automobile engines (too often thought a demeaning activity in Britain), to use computers and sometimes to mend them. But, third, it is a disservice to young people (and their eventual employers) to pretend that there is something about technology, and project-based technology, that is independent of scientific understanding. The British inspectors cannot be faulted on that ground, complaining of some of what they have found in schools that this or that activity fails to provide or to evoke a proper understanding of the underlying science. (A little pedantically, they go on to complain that existing courses on applied science and technology pay too little attention to technology based on chemistry and biology.) The cruellest deception, again apparently growing in British schools, is that technology is antithetical to science, even an alternative thereto.

The inspectors are not guilty on that score, but there is every reason to suspect that too many parts of the British government are guilty of the heresy. The inspectors themselves fall into the trap of saying that "technology needs to be a compulsory part of the curriculum" if all school-leavers are to have some knowledge and experience of it. Presumably, since they consider that technology and science are inseparable, the inspectors also consider that science should be dealt with similarly. But is "knowledge" of technology sufficient, and can "experience" of it be gained except at work (not necessarily as an adult)? The British have a legend, probably untrue but relevant nevertheless, that James Watt invented the steam engine against the memory of the lid of his mother's tea-kettle lifted by the steam beneath. Many of his successors were moved to work in industry by the excitement of seeing Watt's and later machines at work. Similar experiences are possible still, but in different contexts. The present anxiety that technology should be a "compulsory part of the curriculum" unfortunately falls into the standard British trap of supposing that everything important should be taught to young people at school, and then made the basis of an examination. □



Academic boycotts

Archaeologists riven by ban on South Africans

PREPARATIONS for the world congress on archaeology due to be held next September at Southampton, England, have been clouded by a ban on attendance by scientists from South Africa. Some of those now disinclined are complaining that the ban is an infringement of the rules of the Union Internationale des Sciences Préhistoriques et Protohistoriques (IUPPS) under whose aegis the congress is planned and whose constitution says that the union's proceedings shall be open to all. And some of those not affected by the ban who had planned to travel to Southampton next year have written to say that they will not now be attending.

Professor Philip V. Tobias, professor of anatomy at the University of Witwatersrand and one of those affected by the ban, said at the weekend that the ban on the attendance of people from South Africa is the first to his knowledge to have been formulated by academics and enforced against fellow-academics. Usually, he said, obstacles to attendance at international conferences are decreed by governments, although there is now a long list of conferences from which people have withdrawn because they object to the presence of certain others.

The row at Southampton seems to have been forced on the local organizing committee by four separate though not necessarily independent pressures. The local university branch of the Association of University Teachers (AUT) seems to have taken the initiative, telling the organizing committee for the congress that national AUT policy forbids academics who are members of the union from maintaining links with South African academics. The students' union at Southampton, which controls some of the accommodation that the congress planned to use, followed with a threat of direct action. The Anti-Apartheid Movement had also written to more than a score of London embassies, asking them to boycott the congress.

Finally, the Southampton City Council, now under the control of the Labour Party, having expressed misgivings about the possible presence of South African scientists in Southampton next September, formally resolved last month to rid itself of all economic, academic and cultural links with South Africa. This decision appears to have been especially influential in guiding the organizing committee to its decision that membership of the congress should not be extended to those working in South Africa; the city council had earlier agreed to contribute towards the cost of the congress £40,000 in cash together with an unquantified amount of help with

accommodation and hospitality.

The members of the organizing committee seem to have found this summer's dilemma a painful experience. The chairman of the committee, Professor John Evans of the Institute of Archaeology at the University of London, explained this week that he and his fellow members had reached their decision reluctantly and in the belief that, without a ban, the congress would not be held. In reply to Tobias's complaint that the local organizing committee should not have taken a decision to ban South Africans without the agreement of the parent body, Evans said that the local committee had financial and operating responsibility only for next year's congress, but that IUPPS had no funds that might be used to meet the expenses already incurred in the preparations for the congress. He said that those who had announced their intention not to participate would be much missed, but hoped that many of them would change their minds nearer the time, and when they have been given a full explanation of the background.

At least some of the difficulties about the Southampton congress have arisen because of the scale on which it has been planned. The organizers appear to hope

that it will be the largest ever, with participation from a number of developing countries that have not attended previous congresses (held at intervals of five years). It is planned that some sessions of the congress will be held in London. Four insurance companies have provided a bank guarantee of £150,000.

According to the conference office at Southampton, the organizing secretary, Professor Peter Ucko, professor of archaeology at Southampton, "used every possible argument" earlier in the summer to persuade the local objectors to abandon their positions. He said this week that he had commended individual participation in the congress and the need for free speech among academics.

The design of the ban of scientists from South Africa is such as to exclude all those who work at South African institutions, or who are paid by South African organizations. (South Africans working outside the republic are not excluded.) The point is especially ironical as it affects Tobias, who was scheduled to give one of the plenary talks at next year's symposium, and who has a British passport.

IUPPS is not affiliated to the International Council of Scientific Unions (ICSU) but to the International Council of Philosophy and Human Sciences (CIPSH), which is itself a dependant of UNESCO. As a further irony, the governing council of IUPPS includes two South African scientists, Tobias and R.J. Mason (also from Witwatersrand). No doubt these two will have more to say as and when the occasion arises. □

Meanness spells end of GMT

THE chances are now high that the Royal Greenwich Observatory will cease to contribute to the international determination of time later in the year. This is yet another unplanned consequence of the reduction of funds available to British research councils by the continuing application of the government's rigorous cash limits.

The difficulties at Greenwich (which, now, is actually placed at Herstmonceux in Sussex) have arisen because of the decision of the Science and Engineering Research Council (SERC) to concentrate support for astronomy on the observational facilities now available, especially at the observatory at La Palma in mid-Atlantic. The Royal Greenwich Observatory has for several months been primarily a means by which the La Palma operation may be supported, with on-site observation reduced to a minimum. The future of the observatory as an independent organization should be resolved when a further report on the subject is available later in the year.

Hitherto, the Greenwich contribution to the international time service provided by the International Bureau de l'Heure in Paris has been sustained by six atomic clocks, for which no further maintenance

funds have been allowed. The result is that observations with important statistical weight will not be available for transmission to Paris when the clocks fail, as they are expected to do in the months ahead.

This does not mean that Greenwich will lack a standard of time, for it is hoped to operate three caesium clocks as part of the laser ranging programme mounted at Greenwich, for which continuing support is being negotiated by SERC. But the phrase Greenwich Mean Time will thereafter need qualification.

A senior member of the observatory said earlier this week that the prospect that Britain might cease to contribute towards the international standards of time had arisen because SERC is required to make decisions about the disposition of its budget within the strict context of its terms of reference.

Other government departments had declined to take over the responsibility, he said, on the grounds that the provision of a time service rested firmly with SERC as the manager of the observatory. The astronomer said that the consequence was that projects considered "desirable nationally" could be forgotten.

John Maddox

West German technology

Bonn says yes to Eureka and SDI

Hamburg

THE prolonged and often acrimonious discussions about West German participation in the European Eureka project and the US Strategic Defense Initiative (SDI) reached a climax in Bonn last week.

Under the chairmanship of Chancellor Helmut Kohl (CDU), four ministers decided not to appropriate DM1,000 million for the Eureka project. This is what the Minister for Research and Technology, Heinz Riesenhuber (CDU), and the Foreign Minister, Hans-Dietrich Genscher (FDP), who had led the negotiations in Paris, had demanded as initial support over four years for West Germany's participation. At the same time, the Bundestag voted in favour of participation in SDI, although the details have yet to be worked out. Chancellor Kohl, who met President Reagan in New York last week, has informed himself on SDI through his adviser Horst Teltschick (CDU), who visited the United States last month to check the conditions for cooperation on SDI in general. Teltschick, like Josef Bugl, chairman of the federal Enquete-Kommission für Technikfolgenabschätzung (commission for the estimation of the consequences of new

anti-American lines. For SPD, independence from the United States is the objective. This is also why the SPD faction in the Bundestag supports the French initiative for the European shuttle Hermes. In this connection, they complain that the first German space project, based on the German-built but NASA-owned Spacelab, was partly hijacked by the Pentagon, which has arranged for a reduction of the scientific payload in favour of a reconnaissance satellite meant to keep

watch on Nicaragua.

Research Minister Riesenhuber, defeated in his plan to raise extra cash for Eureka by Finance Minister Gerhard Stoltenberg (CDU), will now have to take the money from his ministry's budget. Riesenhuber now says that the West German government can give help with grants to industry only when a project is close to the market. But the projects to be discussed at the Eureka conference in Hannover next week, such as environmental programmes and a European information data bank for scientists, are of general public interest and must therefore be eligible for federal support.

Jürgen Neffe

French research sociology

Research council sweetens mobility

THE notorious unwillingness of French researchers to change their jobs, and in particular to leave research institutes for work in industry, may be broken down by a scheme soon to be announced by Pierre Papon, director-general of the major French research council, the Centre National de la Recherche Scientifique (CNRS). Papon's hope is that research directors will be more willing to encourage researchers to move temporarily to industry when he allows them to replace a seconded researcher by a new person.

CNRS has been battling with immobility since the election of the present government five years ago. People's attachment to their homes is traditional, but it had been hoped that the granting to government researchers of civil service status earlier this year, with the accompanying assurance that a scientist would always be entitled to reclaim his or her job after a spell elsewhere, would break the log-jam and even overcome people's fears that moving would exclude them from promotion opportunities.

But during 1985, only 50 of CNRS's 10,000 researchers have taken leave to work in industry for a year or longer. CNRS acknowledges that there has been a change in the spirit in which French researchers regard industrial contracts; they no longer turn up their noses at industrial opportunities, but instead compete for them. But CNRS now thinks it has identified a previously unsuspected impediment to mobility — the reluctance of research directors to let good researchers go.

Whence Papon's intended sweetener. Laboratories that lose a researcher to industry even temporarily will be allowed to recruit a permanent member to their staff, and may also be allowed to increase their intake of research students. On the face of things, the inducement should be more than enough to have directors ushering their researchers away to industry. Papon is even hoping to make the process still easier by using CNRS salary funds, which are paid over in advance by the government, to help people willing to move with

bridging loans for the purchase of new houses, but there may be legal obstacles to this part of his plan.

Meanwhile, Papon is also hoping to make CNRS into a more self-conscious organization. Despite the growth of public support for research in the past five years, academic studies in science and technology policy have been neglected in France. The omission is especially strange because CNRS supports research in the social sciences and the humanities as well as in hard science.

One of Papon's initiatives is the preparation of a history of CNRS itself to mark its 40th anniversary in 1989. Another is a plan for a colloquium, to be held at Montpellier in January, to which science studies specialists from overseas will be invited, and which will attempt to hammer out a programme of research on the impact of science and technology on regional development, an important issue for the present government of France, which can boast of some success in decentralizing science and science-based industry to the regions.

Papon's explanation of why science policy studies have languished in France centres on the view that French institutions are less willing than their counterparts elsewhere to allow their private operations to be analysed. Jean-Jacques Salomon, director of the Centre Science, Technologie et Société at the Conservatoire National des Arts et Métiers in Paris, one of the few French units of its kind, agrees with Papon but also points to the peculiar difficulties, in France, of building up research teams to work on short-term contracts.

Salomon's unit has only five full-time researchers, and Salomon says he has been pressing the government to give him more support. One problem is that present regulations do not allow him to employ researchers on external contracts for more than a year without offering them permanent positions. Another, Salomon says, is the lack of political will.

Robert Walgate

DAS RHEINGOLD



technologies), advised that West Germany should offer financial cooperation with the United States.

The Bundestag rejected the draft contract proposed by the Social Democrats which included among other things the suggestions of scientists in Göttingen last year, including a complete prohibition of space weapons. In the debate, Josef Vosen, SPD spokesman on science policy, warned the government against "selling off" West German technology and specialists. He alleged that the United States intends to collect precious technology from other countries at low cost, and to use this at a later stage to put pressure on the Eastern bloc.

The Social Democrats clearly favour closer technological cooperation in Europe along the lines proposed by France in the Eureka project. The debate has thus been polarized along pro- and

Indian energy

Fast breeder goes critical

New Delhi

INDIA'S Fast Breeder Test Reactor (FBTR), using what is said to be a new indigenous fuel, went critical on 19 October, making India the first developing country to embark on a breeder programme. Built with French help, the 40-MW (th) sodium-cooled plutonium-fuelled loop-type fast reactor at Kalpakkam near Madras is similar in design to the fast reactor *Rapsodie* at Cadarache in France, but with two major modifications; the £46 million FBTR uses an unorthodox indigenous fuel and can also produce 13 MW of electricity.

FBTR is the third Indian reactor to be commissioned this year. A 100-MW research reactor, DHRUVA, in Bombay and a 230-MW power reactor at Kalpakkam went critical two months ago. The Department of Atomic Energy (DAE) says that with the successful completion of the FBTR project, India has joined the select group of countries that have mastered fast breeder reactor (FBR) technology.

The event also marks the beginning of the second phase of India's three-stage nuclear power development programme. Natural-uranium-fuelled pressurized heavy water reactors such as those in Rajasthan and Madras (four more under construction) constitute the first phase. Plutonium produced in these reactors will fuel fast breeders to be built in the second phase. These FBRs will breed uranium-233 from thorium blanketing the reactor core. In the third phase, FBRs utilizing the ^{233}Th - ^{233}U cycle are to be built and DAE hopes the abundant reserves of thorium (360,000 tonnes) in India will sustain the nuclear power programme for 1,000 years. According to DAE, its breeder programme is essential to India whose uranium reserves will support a nuclear power capacity of only 15,000 MW until 2030 AD.

India's FBR programme began in 1968 with French collaboration with the Commissariat à l'Energie Atomique (CEA) supplying the designs of the *Rapsodie* reactor. Four French companies helped the Indian companies with knowhow on the manufacture of the major components such as the reactor vessel, sodium pump, heat exchangers and control drive mechanism. Most materials for their manufacture came from France under a special French credit of FF35 million.

But work on FBTR has slowed down particularly since the Indian nuclear test in 1974. France, which had earlier agreed to provide highly enriched uranium fuel for FBTR, backed down, so that India was forced to develop its own fuel with a high concentration of plutonium instead of enriched uranium. The FBTR is loaded with a fuel mixture of 70 per cent plutonium carbide and 30 per cent uranium carbide,

thus making it the world's first fast reactor with a plutonium-rich carbide fuel. Reactors operating elsewhere in the world use oxides of 30 per cent plutonium and 70 per cent uranium, and most investigations elsewhere with carbide fuel have been limited to fuels with a low concentration (30 per cent) of plutonium.

The team at the Bhabha Atomic Energy Research Centre (BARC), which has developed and tested the fuel in five years, claim that, despite a lower melting point, it is superior to the traditional oxide fuel. If the fuel fulfils its promise, India will stick to it in future commercial breeders.

BARC is also proud of having indigenously developed the technologies for purifying and handling molten sodium, 90 tonnes of which are required by the FBTR. The exact amount of plutonium in its core has not been disclosed, but all of it is said to have come from the country's stockpile not subject to safeguards. "The way the Indian industry has met the stringent requirements of FBTR equipment is an important pointer to the success of the country's future FBR programme, when larger reactors would be constructed", DAE says. DAE has already made a beginning on the design of a 500-MW prototype FBR that will need 1,800 kg of plutonium, and hopes to build its first commercial FBR in 2000 AD.

Meanwhile BARC has scored another success in a different area of technology; the country's experimental 5-MW (th) coal-based magneto-hydrodynamic (MHD) generator at Tiruchirappalli, in south India, started producing 100 kW of electrical power in mid-August. For the past 10 years, the Moscow high-temperature institute has been helping BARC and the state-owned Bharat Heavy Electricals Ltd in the setting up of the MHD pilot plant, which uses high-ash Indian coal. Water-gas obtained by passing steam over red hot coal in a combustor is used as the feed gas, which is seeded with potassium iodide. The combustor and the 300-tonne iron core magnet that can carry up to 1,300 amperes without water cooling were designed by BARC.

The test run in August, which lasted for two hours, produced a plasma at 2,800 K, 100 kW of power at a Faraday voltage of 5 volts and a 0.7-tesla field. The Soviets, whose MHD plants are fuelled by oil or natural gas, had much to learn from the operation of the coal-fired Indian MHD plant. The basic data had helped the Soviet Union build a 25-MW (th) coal-based MHD plant from which extraction of up to 5 MW of electricity has already been achieved. India, which has no plans to scale-up its pilot plant, has declared it a national facility for studying properties of glass and ceramics at high temperatures.

K.S. Jayaraman

Romanian energy

Too soft on soft energy

ROMANIA'S Minister of Electric Power Supply, Nicolae Busui, was dismissed two weeks ago, and the electricity generating industry placed under military control, because of inefficient management and failure to achieve the planned output. Under the emergency regulations, all coal-fired power stations will be placed under a military commander, who will be responsible for the "strict observance of technological, exploitation and maintenance norms" as well as for order and discipline. Power workers are forbidden to change their jobs, and all vacant posts must be filled from outside, not by transfer within the industry.

Romania, formerly a net energy exporter, has for almost a decade suffered from a growing energy crisis culminating in the virtual shut-down of the country during last winter's cold snap. During the 1970s the over-expansion of the petrochemical industry (largely on the initiative of Dr Elena Ceaucescu, wife of the President) decreased indigenous petroleum supplies, with the result that thermal power stations are, for the most part, coal fired. The official statistics carry glowing reports of record coal and lignite outputs and of deliveries ahead of schedule to the power stations, so that what has gone wrong at the generating plants is far from clear; there appear to have been considerable delays in the servicing of existing stations and the installation of new ones, but such delays are endemic throughout the Romanian economy.

To some extent, the coal-fired generating plants may have been made the scapegoat for other sectors. The nuclear power station at Cerna Voda, for example, in spite of a recent, much publicized speed-up, is well behind schedule. So too are plans for a network of hydroelectric plants with a total generating capacity of 13,000 MW.

The emergency decree urges modernization and technological innovation in the Romanian power industry. In fact, the Romanians are prolific in technological novelties, including wind-powered turbines, wave power, solar power and biogas. In 1984, under a programme coordinated by the National Council of Science and Technology on unconventional and renewable energy, facilities equivalent to about 115,000 tonnes of conventional fuel were put into operation, while the planned equivalent this year is 214,000 tonnes. How much of the planned investment in the generating industry (37,500 million lei this year) goes on these unconventional projects is unknown, but the emergency suggests that more attention should be paid to the conventional sector.

Vera Rich

UK research councils

Hatchet buried for time being

THE Royal Society and the Natural Environment Research Council (NERC) appear for the time being to have patched up their differences on the support of geophysics research in Britain. That, at least, is the simplest reading of a statement put out this month by NERC and the society describing, in language usually reserved for diplomatic communiques, a meeting between the two sides which is said to have taken place in late August.

The occasion for the meeting was the publication in June (see *Nature* 315, 709; 1985) of the report of a working party on geophysics research commissioned by the society and endorsed by the society's

council. The working party, under Professor R.E. Oxburgh, complained of the generally inadequate scale on which geophysics research is at present reported. In passing, the committee made several criticisms of NERC's management of research support in the field, drawing from the research council's chairman, Mr Hugh Fish, the response that he "took great exception" to the report, and that he would seek a meeting with the Royal Society.

That meeting is now said to have examined "a number of differences of view" and to have included a "full and frank discussion of a wide range of issues". The statement says that the "Royal Society learned with interest and much satisfaction" that several changes in NERC management practice had taken place since the working party began work. The statement says that the society and the research council will work together to forge "an increasingly constructive relationship" between the scientific community and "one of its major sources of financial support". The two bodies unite, in the concluding paragraph of their statement, in saying that "many of the difficulties were largely" consequences of the reduction of NERC's budget in recent years.

The formulae in the published statement appear the basis for at least a temporary truce. NERC, meanwhile, is quick to point out that many of the criticisms raised by the working party have been met by changes already under way before the appearance of the report. Thus steps have been taken to rationalize the allocation of time on research vessel cruises (which, the Oxburgh group said, had often been cancelled at short notice, and which were complicated by the circumstance that the committee responsible for allocating research time was different from that responsible for research grants to make good use of the time). Similarly, NERC says, steps were already under way to decentralize the computing services network, which NERC researchers have been required to use but over which they have had no management control.

NERC's plans for a new management structure appear to be making progress, if slowly. The proposal, in the "corporate plan" published last year, that the council's research institutes should be grouped together under three newly appointed scientific directors has so far led to the appointment of Dr Bernard Tinker, deputy director of the Rothamsted Experimental Station, as the one in charge of the council's environmental work. The search continues to fill the two other vacancies.

Some progress has also been made towards a solution of the problem of the British Geological Survey, the largest single component of NERC's annual

budget cost — or, at least, a committee has been set up. Jointly with the Advisory Board for the Research Councils, NERC has commissioned a study from a group whose chairman is Sir Clifford Butler, vice-chancellor of Loughborough University of Technology, and which includes Sir Alan Muir Wood, Dr Charles Suckling, Sir Alwyn Williams and Sir Frederick Warner. Of these, only Williams is primarily an earth scientist, although Warner was the chairman of the recent SCOPE-sponsored study of nuclear winter.

The terms of reference for the new study are wide enough to allow quite radical recommendations. The hardships that afflict the survey, fully documented in the past year, have arisen because government departments have gone back on an agreement more than ten years ago to contribute towards the cost of the survey, but there is a body of opinion now strongly represented among members of the survey that the British government should make a commitment to the continuation of the systematic surveying of British geology. □

New law for West German universities

Hamburg

DESPITE strong opposition, the government's amendments to West Germany's *Hochschulrahmengesetz* (university regulations) have now been passed by the *Bundstag* (parliament). The changes have provoked much controversy since they were first announced by the Christian Democrat Education Minister, Dorothee Wilms, earlier this year.

In June nearly 40,000 students took part in demonstrations against the new laws (*Nature* 316, 96; 1985) and during the parliamentary debate the students' objections were raised. The opposition parties — the Social Democrats and Greens — voted against the changes, which bind the regional governments (*Länder*) to implement the new regulations during the next few years. During the debate all parties voiced the opinion that more opportunities should be made available to women scientists. But the opposition was critical of the government's position, saying that a mere declaration of intent will do nothing to improve their lot.

Whereas Wilms welcomed the new law as a "landmark for the political development of the federal universities", the opposition parties are still against the introduction of a two-tier system of studies, whereby specially talented students are allowed to take extra courses but the majority of students have to complete their studies after six semesters.

The proposed relaxation of rules covering industrial research is also unpopular. The government claims, however, that the newly created post of "scientific assistant", which replaces the old "university assistant" will help to strengthen the position of the rising generation of students. The legislation faces one last hurdle, the *Bundesrat*, which is the representative body of the *Länder* but where the federal government has a majority.

Jürgen Neffe

Japanese ibis saved?

Tokyo

A FRESH effort to save *Nipponia nippon* (the Japanese crested ibis or *toki*), from extinction began this week with the arrival of one bird from Beijing Zoo at Japan's ibis protection centre on the island of Sado. The *toki* is now one of the world's rarest birds, with only three remaining in Japan and perhaps as many as twenty in China.

In the nineteenth century, the *toki* was a common bird of paddy fields and ponds in many parts of east Asia. Its white plumage, somewhat comical red face and heavy downward curving black bill made it a popular subject for painters. But despite protection measures that began in Japan in the 1930s, numbers declined so rapidly that all five remaining birds were placed in captivity in 1981. Since then two birds have died but several more birds, including three chicks, have been discovered in China. But because the area in which they live is known to be polluted with mercury and manganese from chemical fertilizers, there are doubts as to how long they can continue to survive in the wild. Thus a cooperative programme to breed the *toki* in captivity has begun.

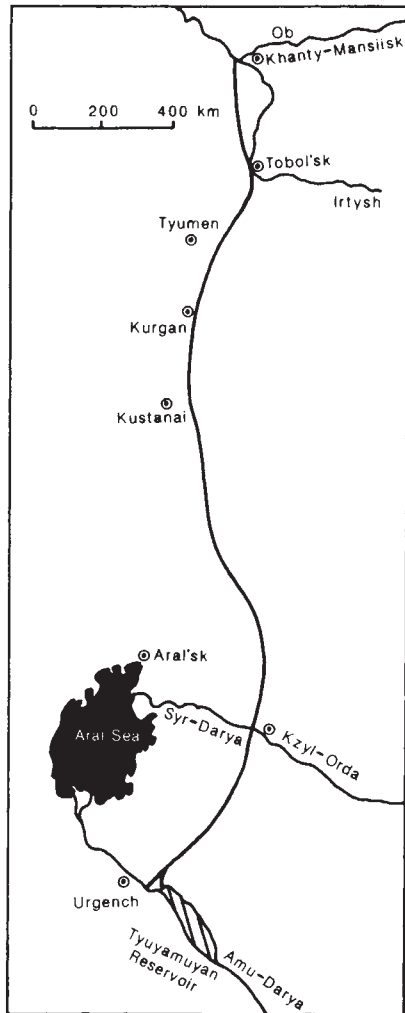
The Beijing bird flown in by chartered plane is a young male, named Hua Hua, which will replace Japan's one remaining male, now thought to be too old to mate. But Hua Hua is likely to find his work cut out. *Kin*, the one female in good condition, would be in her eighties if measured in human terms and, having been brought up in captivity, has never reared young. Hua Hua will remain in Japan for three years and meanwhile research on artificial insemination at the ibis centre will continue.

Alun Anderson

Soviet rivers

Ambivalently flows the Ob

SIBERIAN rivers, a favourite target of grandiose planning since the early days of Soviet rule, are no longer destined to "run backwards". Instead, a carefully calculated "part of the flow" will be channelled southwards, after possible "climatic and ecological changes" have been taken into consideration.



Massive hydroengineering projects, involving the imposition of the planners' will on nature, seem to appeal to the Soviets. In the 1920s, the need for such schemes was enthusiastically espoused by the propagandists of atheism on the grounds that, if God existed, He would have designed Siberia more rationally. In the 1930s, the construction of the White Sea Canal absorbed thousands of the victims of Stalin's purges and could be explained, if necessary, abroad, as "reeducation through labour".

In the early 1970s, the need to divert the Siberian rivers was used as an argument for peaceful nuclear explosions (an option since abandoned by treaty). But the international outcry in the 1970s against the possible effect of such diversion schemes on the Arctic, and indirectly on the weather worldwide, evoked, first of all, Soviet

assertions that all ecological factors had been taken into consideration by the planners, and then somewhat later the launching of a major research project into the ecological consequences.

The inauguration of the long-term "Food Programme" in 1982 (the last major policy decision of the Brezhnev era) once again emphasized the need for a grandiose irrigation project in Siberia. (In fact, irrigation is needed not only for food crops but also for cash crops such as cotton, but Brezhnev's team emphasized the food aspect.) At the same time, the name "Sibiral" first came into common use.

A map of the proposed "Sibiral" canal was published only last August, in *Pravda Vostoka* (Eastern Trust), a paper not normally accessible in Moscow, let alone abroad. Even here, very few details were given. While the accompanying article spoke glowingly of the four great pumping stations needed to lift the water to the Tyumen' watershed, the map gives no indication of where these will be sited. It merely traces the proposed route of the canal at an undefined distance to the east of Tyumen', Kurgan and Kustanai, crossing the Syr-Darya somewhere to the west of Kzyl-Orda, and entering the Amu-Darya below the Tyuyamuyan reservoir east of Urgench.

Even more significantly, the *Pravda Vostoka* article reveals for the first time that there has been a major disagreement over the route. Since irrigation plans for Central Asia and Kazakhstan indicate that by 1987 the waters of the Syr-Darya and by 1995 those of the Amu-Darya will be totally consumed, there is every need to press ahead with the Sibiral, and in October 1984 the Plenum of the Central Committee of the CPSU brought the completion date forward to 1987. The task involves 6,000 million m³ of earthwork and 15 million m³ of concrete construction, to produce a canal 2,600 km long, 250 m wide and up to 12 m deep, with a flow of water of up to 1,150 m³ per s. Moreover, the project appears to have been held up by a dispute between the planners and the scientists. According to *Pravda Vostoka*, 23 institutes of the Soviet Academy of Sciences and around 130 other research and design institutes have been involved in planning the Sibiral. Under their pressure, the planners have moved the canal route an unspecified distance eastward of the original track, where, it is claimed, it will have "no negative effect" on traditional agricultural systems, nature reserves and ancient forests, and will preserve animal migration routes. As well as pleasing the ecologists, the eastern variant has the support of the economists — the terrain, according to *Pravda Vostoka*, has considerably reduced the estimated construction and utilization costs. **Vera Rich**

Biosafety regulations

US at odds with OECD

Washington

THE Commissioner of the Food and Drug Administration (FDA), Dr Frank Young, has been strongly criticized by two US congressmen for failing to resolve an inter-agency dispute that is putting in jeopardy an international study attempting to develop uniform approaches to biotechnology regulation. A completely rewritten draft of the study report, produced by FDA, ignores agreements reached previously with other member countries of the Organisation for Economic Cooperation and Development (OECD), according to Representative John Dingell and Senator David Durenberger.

The OECD report should have been completed in June this year, but at a meeting in May in Paris the United States completely reversed its earlier approach, leaving other delegates wondering whether it was trying to scuttle the project. The report had been two years in preparation, under the chairmanship of Dr Roger Nourish of the United Kingdom's Health and Safety Executive.

Until the May meeting, the US delegation had been led by the Environmental Protection Agency (EPA). But FDA's biotechnology policy coordinator, Dr Henry Miller, was put in charge of the delegation after voicing strong objections to the direction the study was taking under EPA. Miller was actively opposed by EPA delegates in Paris, and the delegation "completely disintegrated", according to one observer.

The highly-charged quarrel between FDA and EPA mirrors an earlier dispute between FDA and the National Institutes of Health over guidelines for human gene therapy, which FDA lost. Young, and through him Miller, lost some political kudos when Margaret Heckler agreed to step down as Secretary of Health and Human Services, and FDA's hopes of leading biotechnology policy-making are now open to question.

The May draft of the OECD study, produced when EPA was still in charge of the US delegation, devotes much space to descriptions of standard levels of containment and lays much store on flexibility, but gives no indication of how knowledge about possible hazards should be used to decide on appropriate containment levels. It lists only information that should "ideally" be taken into account. Some reviewers criticize it for being prescriptive, although almost the only firm recommendation is that viable engineered organisms used in industry be kept in closed systems. Most agree that the study was seriously flawed; opinions vary over whether it was rescuable.

The new FDA-inspired September

draft, called *Safety considerations for commercial and environmental applications of organisms derived by recombinant DNA*, is so different as to be unrecognizable. In general, it plays down risks, and declares that its aim is to present "the maximum number of approaches a country might choose" to deal with safety considerations. It observes that "it is widely accepted that the hazards associated with recombinant-DNA-containing microorganisms may be assessed in the same way as those associated with other organisms", and concludes that "there is no scientific justification for singling out rDNA work for a special regulatory regime above that which is already established" for traditional biotechnology such as brewing. Residual concerns about predictability of field trials of modified organisms have been expressed "primarily by ecologists who generally possess no direct experience with rDNA or related techniques" and represent a minority opinion.

Dingell and Durenberger have written to Secretary of State George Schulz to ask that he get the OECD study "back on track". Many OECD countries are keen to have international authority for their regulatory approaches as soon as possible, and the 4-month delay brought about by the US shift has caused considerable irritation. But unless the FDA September draft is accepted, further delays are in prospect.

Tim Beardsley

US-China nuclear deal

Washington

THE odds against congressional ratification of the nuclear technology pact between the United States and China became slightly greater last week, when Senator Alan Cranston, the Californian Democrat whip, threw in his lot with opponents of the scheme. Cranston believes that China is exporting nuclear technology to Brazil, Argentina, Iran, South Africa and Pakistan, the countries with the "most dangerous" nuclear programmes, despite China's pledge not to provide others with the means to develop nuclear weapons. He also asserts that Richard Kennedy, negotiator of the agreement, has deliberately withheld this information from Congress.

China acknowledges that it has peaceful nuclear cooperation agreements with Pakistan and Brazil, but denies a similar relationship with Iran. A statement from China's foreign ministry in response to Cranston's comments says that China's nuclear cooperation with any other country serves only peaceful purposes.

Cranston will help John Glenn (Democrat, Ohio) and others to draft legislation making China's acquisition of US nuclear technologies more difficult without firmer assurances on its non-proliferation policy. But Cranston admits that the chances of Congress rejecting the pact are still slim.

Maxine Clarke

US data protection

Privacy bill progresses slowly

Washington

MODERNIZATION of regulations on the privacy of electronic communications moved further forward last week, when representatives of the industries concerned endorsed the proposed legislation with only a few minor quibbles. The Electronic Communication Protection Act was introduced to both houses of Congress last month, and there will be a series of hearings during the next few months before the final version is drafted and voted on.

To coincide with last week's hearings, the Office of Technology Assessment (OTA) released a report, part of a study of the effects of new information technology on the federal government, which concludes that the technological revolution of the past 20 years has far outpaced laws to protect civil liberties. OTA's survey of 142 federal agencies shows that about 25 per cent of them use, or plan to use, electronic surveillance and that 25 per cent use computerized records.

The "wiretap" law of 1968 made telephone calls private, but the dramatic changes since then in communication — electronic mail, cellular and cordless telephones, radio and video surveillance — have no associated statutory protection. OTA's report spells out how information technology is affecting electronic communications, but does not grasp the nettle of the national security agencies' use of the technologies.

The new act would extend protection to virtually all electronic communications, eliminating the distinction between common and private carriers. It would also protect against unauthorized disclosure of third records, regulate the government's use of pen registers and tracking devices and create penalties for illegal access. But the difficulty is freely acknowledged of allowing for future development in an area that attracts the most daring entrepreneurs, and which is thus liable to explosive development.

Fred Weingarten, one of the authors of OTA's report, says nevertheless that the bill is an "important effort at addressing the potentially significant threat to the traditional privacy of communication". One of the quickest ways for an information service company to go out of business is to abuse the privacy rights of customers, says Michael Nugent of ADAPSO, the trade association for the US software and services industry. ADAPSO and the Telelocator Network of America, the national association of radio common carriers that produces cordless telephones and radio paging services, gave their support to the bill at last week's hearings.

Representative Robert Kastenmeier (Democrat, Wisconsin) and Senator Patrick Leahy (Democrat, Vermont), co-sponsors of the bill, hope to encourage

the information technology market by assuring customers of protection as well as by providing comprehensive statutory protection from unauthorized surveillance. Until Congress passes some form of legislation, interim decisions have to be made in the courts, a time-consuming, expensive and unsatisfactory process.

It is to be hoped that the US law, when passed, will avoid the vagueness that has caused so much controversy over Britain's Data Protection Act, which has been widely criticized for the scope it provides for invasion of civil liberties (see *Nature* 307, 494; 1984).

Maxine Clarke

Texas technology

Washington

TEXAS, the Lone Star state which was briefly last century an independent republic, is once again displaying secessionist tendencies, this time in the more limited domain of scientific research. The state legislature has recently awarded 87 grants worth \$35 million to Texan colleges and universities for scientific and high-technology research likely to bring economic benefits to the state.

The Texas Advanced Technology Research Program was the initiative of state lieutenant governor William Hobby. Its approval was all the more surprising because Texas this year faced a budget crisis due to falling oil revenues, and some unheard of (for Texas) austerity measures were introduced. Supporters of the scheme argued, however, that falling revenues were all the more reason to support research of the kind likely to generate "practical sorts of things" for the state.

Support was provided for seven principal areas of research: aerospace, agriculture, biotechnology, chemistry and physics, energy, microelectronics and telecommunications, and materials science. Decisions on which applications to support were made by a panel of experts appointed from outside the state, headed by Dr Frederick Seitz, who among other things chairs the advisory committee to the Strategic Defense Initiative. When Seitz asked whether applied or basic research should be favoured, he was told just to choose "the best", according to a state official. The University of Texas and Texas A&M University were the main beneficiaries.

Texas claims its scheme is unique in the country. The initial \$35 million is for two years, but the scheme will be continued if all goes well. It has, according to the Texas official, received the blessing of the National Academy of Sciences, whose chairman, Dr Frank Press, is said to regard it as a prototype of what might be achieved elsewhere.

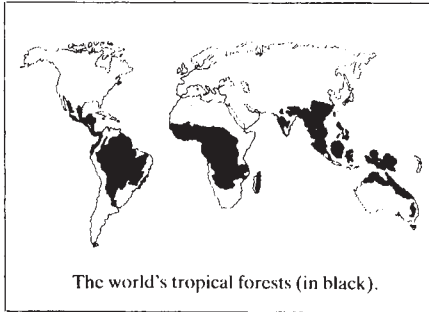
Tim Beardsley

Deforestation

Lending agencies plan action

Washington

THE major international development assistance agencies have for the first time reached a consensus on how to tackle the problem of deforestation. Not only that, but an impressive collection of environmental lobbying groups support the plan, among them some of the severest critics of the agencies' lending programmes.



The world's tropical forests (in black).

One reason for the harmonious outcome is the need to persuade hard-nosed politicians, and not just the environmentalists, that the problem is severe enough to demand enormous investment programmes. Gus Speth, president of the World Resources Institute (WRI), likens the global deforestation crisis to the oil crisis faced by industrial countries in the 1970s.

Another reason for the general acceptance is the explicit acknowledgement that the individual countries concerned accept responsibility for helping to solve the problems by encouraging small, rather than grandiose, projects.

The plan itself, whose main sponsors are WDI, the World Bank and the United Nations Development Programme, calls for a five-year investment of \$8,000 million to reverse global tropical deforestation and its environmental effects. To prevent the loss of 27 million acres of forest each year from the developing countries, the plan "allocates" \$530 million to 56 Asian, Latin American and African countries, half of which would be supplied by international lending organizations and half by private investors and the governments of the countries concerned. Large as these figures are, the report stresses that they are small compared with the costs of crisis management. For example, a largely ignored United Nations programme in 1977 recommended that \$50 million a year be spent to avoid desertification in Ethiopia; the report estimates that the Ethiopian famine has since cost \$400 million in relief programmes, to which at least \$100 million will be added before the next harvest.

The international task force is confident that it can influence global tropical deforestation, although Speth admits that "we have a distance to go in getting firm pledges of exact dollars" from lending

agencies. According to John Spears of the World Bank, the "will exists" to lend but support depends on the participation of individual governments, an opinion echoed by other sponsors of the report.

The report will be presented to the United Nations Commission on Environment and Development in Brazil this week; next month international development assistance agencies meet in the Hague to discuss it. Negotiations are well advanced for a summit meeting "at the highest level" early in 1986. **Maxine Clarke**

Tropical Forests: A Call for Action WRI Publications, PO Box 620, Holmes, PA 19043, \$12.50 (1985).

Big fish love little fish

Washington

Two recent acquisitions of start-up biotechnology companies by established pharmaceutical houses may signal the start of a new trend. In September, Eli Lilly and Co. announced its plan to buy Hybritech Inc. of San Diego for over \$300 million, and last week the Bristol-Myers Company announced an agreement in principle to buy Genetic Systems Corporation of Seattle for \$294 million.

The two deals have a number of similarities. Neither Hybritech nor Genetic Systems was facing a cash crisis, but neither has yet recorded profits. More important, both are hoping to move from reliance on diagnostic products to therapeutics, which are said to cost on average \$100 million to develop and steer through trials before they receive market approval.

Hybritech already has several diagnostics based on monoclonal antibodies on the market, including pregnancy and cancer tests. It stands to gain much from Lilly's financial and technical resources, as well as marketing knowhow — Lilly is already selling the first human recombinant DNA-derived pharmaceutical, a synthetic insulin.

Much the same can be said for Genetic Systems and Bristol-Myers. Genetic Systems has one diagnostic product on the market, a monoclonal-based test for Legionnaires' disease. Also in the pipeline are HLA cell-typing and tissue-typing reagents, and diagnostics for hepatitis B and acquired immune deficiency syndrome.

It is as yet unclear how the acquisition by Bristol-Myers will affect a previous unconfirmed investment plan by Syntex Corporation, which is a part owner of Genetic Systems. But the deal is not expected to affect Oncogen, an offshoot of Genetic Systems devoted to cancer research and owned jointly by Syntex, Genetic Systems and Bristol-Myers. **Tim Beardsley**

Contraceptive pill

Japan heads for legalization

Tokyo

JAPAN is just coming around to considering the legalization of the contraceptive pill, some twenty years after other major nations. Indeed, with the pill now in use in 150 countries, Japan must be almost the last non-Catholic country and certainly the last industrialized country, in which the pill has been prohibited.

What has brought about this strange state of affairs? Earlier this year Singapore's Prime Minister, Lee Kuan Yew, praised Japan for keeping the ban on the pill as it had helped to retain "Asian values", including "chastity and a high level of fidelity, and the integrity of the family . . .". But officials of the Ministry of Health and Welfare were quick to point out that the pill has been banned only for fear of possible side effects.

That is something of an overstatement. Moral considerations certainly counted when the pill was originally prohibited nearly twenty years ago. But paramount consideration went to the pill's possible side effects, Japan having just experienced thalidomide poisoning and other cases where drug companies found themselves faced with demands for huge compensation payments. Curiously, it now turns out that Japan's attempt to avoid the pill's side effects have rebounded.

Two organizations, the Japan Obstetrics and Gynaecology Society and the Japan Motherhood Protection Medical Association, have now asked the Ministry of Health and Welfare to give permission for clinical tests to be made of the pill. One of the key reasons for their demand is that the pill has actually come into fairly common use. Although doctors are not permitted to prescribe the pill for contraceptive use, they may prescribe it as a medicine, for treatment of irregularities in menstruation, for example.

Using this regulation as a loophole, many doctors now openly provide the pill as a contraceptive. The trouble is that they can prescribe only the high-oestrogen formulations designed for medical rather than contraceptive use. The low-dose pill in use in the United States and Europe is still unknown. The many women who take the pill in Japan thus run a far greater risk of side effects such as thrombosis, than if it had been legalized in the first place.

The Ministry of Health and Welfare has taken the new request positively, but it is not yet clear what action will result. Fresh clinical trials would have to be performed in Japan, even for products in widespread use elsewhere and these are expensive, perhaps costing 3,000 million yen. Any pharmaceutical maker wanting to put a product on the market would thus find itself faced with a very large bill that might

not be commercially justifiable. A further pressure comes from doctors in the abortion business, who have consistently opposed the pill; for them, abortion services provide a cash income that would be hard to replace. There may well turn out to be a temptation to leave matters as they stand, to the detriment of those taking a high-dose pill.

Alun Anderson

Planetary science

NASA decision causes dismay

Washington

THE decision of the National Aeronautics and Space Administration (NASA) to abandon the Comet Rendezvous and Asteroid Flyby (CRAF) mission as a candidate for a new start in fiscal year 1987 has caused dismay to planetary scientists on both sides of the Atlantic. The mission was to have been the first using the Mariner Mark II Spacecraft, now under development, and there is concern both about the budgetary implications for planetary science and about planning of future missions that may benefit from information provided by CRAF.

NASA's decision resulted from budgetary pressures; the two new starts it has requested for fiscal year 1987 are TOPEX, an oceanographic satellite, and the first part of the US contribution to the International Solar Terrestrial Physics Program. Starting planning for CRAF in 1987 would have meant the agency could look forward to a launch in 1992. The enforced delay makes it likely that a group of engineers working on the Galileo Jupiter spacecraft, now nearing completion, will have to be disbanded rather than moved straight to CRAF, and has shaken the faith of those who expected NASA to continue supporting planetary science to the tune of about \$300 million a year.

The delay is also likely to impede planning of future missions that might involve collaboration between NASA and the European Space Agency (ESA). ESA is thinking about a sample/return mission to a "primordial body" (asteroid or comet) some time in the next decade that might use CRAF data, and there has been talk about plans for NASA/ESA collaboration on a mission to Saturn, called Cassini, that would, like CRAF, have been based on the Mariner Mark II craft. West Germany is expecting to fly scientific instruments and provide a propulsion module for CRAF, and the decision has caused "great disappointment" there according to one researcher. Dr J. Kissel of the Max Planck institute for nuclear physics said West German scientists would have valued more time to prepare their instrument proposals if the mission is to be delayed on NASA's account; as things stand, NASA is expecting formal proposals before the end of this month.

Tim Beardsley

British astronomy

Lockyer observatory to be sold

THE future of a trust established by Sir Norman Lockyer, the editor of *Nature* from the foundation of the journal in 1869 until his retirement after exactly 50 years, for the benefit of British astronomy is now in doubt. The council of the Norman Lockyer Corporation, the company limited by guarantee which for the time being owns the 44-acre site at Sidmouth, in East Devon, on which Norman Lockyer's observatory is situated, is on the point of selling the largely decrepit collection of telescope domes and buildings to the East Devon District Council for £140,000.

An attempt by Mr Norman Walker, an astronomer at the Royal Greenwich Observatory (RGO), to rescue the Sidmouth site is meanwhile in limbo. Mr Walker has been negotiating with the executive council of the Norman Lockyer Corporation, and latterly with the putative owners, the East Devon council, on a plan to refurbish one of the telescopes and to install a 100-inch reflector in another of the three telescope domes. Mr Walker says that if he could lease the site from one or other of its owners at a reasonable price, he would give up his job, move to Sidmouth and hope to pay for the cost of maintaining the essential buildings on the site by the income from an estimated 50,000 visitors a year to an exhibition he would mount.

Mr Walker's scheme is only the latest in a series of attempts to rescue the Sidmouth scheme in the past two decades, since the Norman Lockyer Corporation formed a loose association with the University of Exeter (then the University College of the South-West) in the heady days of the expansion of the British university system. As things are, there are no funds (and no ambitions) to support a programme of observational astronomy at Exeter, while the cost of keeping the site in some state of order is beyond the resources of the university. A scheme for turning the Sidmouth site into an educational centre for East Devon schools, canvassed a decade ago, similarly came to nothing.

According to Mr M. J. Hislop, the registrar of the University of Exeter who is secretary of the Norman Lockyer Corporation, negotiations with the East Devon council have been under way for longer than the two years during which Mr Walker's proposals have been put forward. The proposed selling price for the Sidmouth site is apparently that recommended by the district valuer, a person whose statutory duty is to value property for property taxation purposes. Mr Hislop says that the site is covered by restrictions on development which reduce its value.

Nevertheless, Mr Walker says he is offended that the proposals put to him by the subcommittee appointed by the East

Devon council to look after the proposed purchase are unacceptable. Apparently he has been offered a lease on the part of the site occupied by the three domes and the three other buildings (a workshop, a set of offices and a bungalow) for £47,000, with a nominal rent for the first few of 90 years, but with the provision that he would not be able to transfer the lease to the handful of colleagues who he hopes will join in the planned flight from RGO to what he hopes will be one of the few remaining sites for observational astronomy in Britain.

The corporation's view of the future, according to Mr Hislop, is that the sale to the local council will now almost certainly go ahead, although a decision to dispose of the only substantial asset of the charitable trust would have to be agreed by a meeting of the whole corporation, which appears not yet to have been arranged. Whether Mr Walker can back his plans with firm undertakings of financial support before that meeting is an open question.

One curious feature of these developments is that Lockyer seems to have been over-exact in his design of his charitable trust, whose objects are said to be "to build and maintain observatories for the good of astronomy". The result will be that if the proposed sale goes ahead, the corporation will either have to find some other cognate project on which to spend £140,000 or arrange that the terms of the trust are changed.

Mr Hislop says that while the membership of the corporation includes some who were members in Lockyer's time, it now also includes many who have joined to "find out what is going on". □

Weizmann Institute

Rehovot

IMMUNOLOGIST Michael Sela, president of the Weizmann Institute for the past ten years, will step down at the end of the month to be replaced by mathematician Aryeh Dvoretzky, now head of the Institute of Advanced Study at the Hebrew University. Sela plans to return to full-time research.

Sela is proudest of his programme of career development chairs, usually endowed by specific donors, by means of which 60 younger scientists have so far benefited. Building work has been given low priority, chiefly because of declining support from the government of Israel.

Best known for his work in synthetic antigens, Sela intends to use a sabbatical year in the United States to develop his interests in synthetic vaccines, multiple sclerosis and the immunological targeting of drugs.

Nechemia Meyers

Why down with metric?

SIR—Using as a pretext the story of a computer error in the satellite orientation, D.C Jolly launches a harsh attack on the metric system (*Nature* 316, 480; 1985). Attempts to slow down the gradual metrication of the United States are hardly understandable in view of the benefits the metric system inevitably brings to any country adopting it.

Why did Japan during its rapid modernization in the late nineteenth century adopt the metric system (still little known at that time) and not the imperial or American system? Most other countries did the same including Russia after the 1917 revolution.

The metric system peacefully conquered almost the whole world. Its obvious convenience and progressiveness stems not from the French government decree of 1840 but from its inner accord with the way we count and from the entire commensurability of all metric units. We have 10 fingers and not 12 and this is why our numerical system is based on 10 and not on 12, despite the fact that 12 can be divided by 2, 3, 4 and 6 while 10 divides by only 2 and 5. One can ask if God or Nature made a mistake at this point, but this is the fact we have to live with!

In sharp contrast with the metric, the traditional English (and slightly different American) system of weights and measures has very poor inner self-consistency. It is not even based on the power of 12: while there are 12 inches in a foot, the inch itself is traditionally divided into 16 and not 12 parts. There are 16 ounces in a pound, 3 feet in a yard and 8 pints in a gallon. The two main units of length have no common basis at all—it is very difficult to find any meaning in the number 5,280 which is the ratio of a mile to a foot and which is, of course, remembered by almost nobody. The list of oddities goes on and on. The gallon, for example, is nicely equal to 277.3 cubic inches and an acre amounts to 43,560 square feet (!). Even leaving aside the US gallon versus imperial gallon, statute mile versus nautical mile and regular ounce versus troy ounce, how can anyone claim the superiority of all this jumble over the system based on the simple decimals? It is symptomatic that the units of the imperial system, lacking any common basis, are defined through their metric equivalents (legal definitions of foot, pound etc. in the United States).

There are many things in the world that divide nations and people. The metric system (along with the Western calendar) is one of the very few instruments that help to unite us.

The future belongs to metric. Science is already metric worldwide (including, of course, the United States) and so is any scientifically based technology. It is well known that the delay in metrication was

one (although not the main) reason for Britain's loss of economic position after the Second World War. Some US industries already prefer to use metric in their operations. We just cannot afford not to be metric.

Almost all progressive innovations have been opposed in the past and often very violently. People resisted printing, telescopes, steam engines, railways, telephones (invasion of privacy), cinematographs (produce mental disorders), automobiles, blood transfusion and use of X rays in medicine. Now some resist nuclear science, cosmic studies and molecular genetics (expensive and dangerous). The opposition to the metric system just belongs to the same category. In other "hot topics" of public dispute today (abortion, star wars and so on) both sides have at least some argument to support their positions. But there is indeed nothing behind the imperial system except stubborn resistance to progress mixed with ignorance. It affords no honour to a nation to insist on the obsolete. Nostalgic superstitions are of no help to economic and social progress.

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Freewill and entropy

SIR—Professor John Searle, in the final lecture of the Reith Lectures for 1984, was unable to justify freedom of the will. According to Professor Stuart Sutherland (*Nature* 313, 163; 1985), "if the mind is merely the brain under another guise, and if the physical world is determined, then there appears to be no room for freedom of the will".

The *Oxford English Dictionary* (OED) defines freewill as "the power of determining one's choice of action independently of causation or fate", but is rather too optimistic, given the realities of life; certainly there are goals that may not be achieved by most people, despite the greatest of ambition. But the concept of freewill in the philosophy of life is definitely more attractive to the independent thinker than that of determinism, defined by OED as "the doctrine that human action is not free but determined by motives regarded as external forces acting on the will".

Freewill implies divergent thought, while determinism implies convergent thought processes. The scientist must be capable of divergent thought when arriving at an hypothesis and then must converge on the salient features in establishing whether the hypothesis is true or false.

As far as determinism is concerned, it is generally agreed that every effect has a cause or causes. This is a necessary condi-

tion for determinism but is not sufficient. For sufficiency, the causes must be determined. But this sufficiency may be unattainable. For all processes, the second law of thermodynamics gives $\Delta S > 0$ where S is entropy.

In all but the most idealized process, the entropy of the Universe is increasing and every cause has the effect of increasing the disorder of the Universe. Causes are lost in this increase of entropy and are indeterminate as far as predicting the future is concerned. Hence, in living systems, determinism will never catch up with events which we may therefore interpret as the results of freewill.

Consciousness may be interpreted as the manifestation of biochemical processes as electrical activity. But the firing and switching of the neurones combined with the release and binding of the various neurotransmitters at the synapses are processes which ensure that the brain functions far from thermodynamic equilibrium. In these circumstances, entropy production makes it impossible for the brain function of freewill to be determined.

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Data in dock

SIR—I wish to draw the attention of your readers to the unfortunate circumstances that may arise when erroneous scientific conclusions are published in the technical press.

In 1967, in a letter to *Nature* (216, 83; 1967), Dr Isabel Gal published evidence purporting to show an association between the occurrence of neural tube defects (meningomyelocele or hydrocephalus) and the administration of hormonal pregnancy tests to the mothers; Dr Gal, indeed, advocated the hypothesis that exogenous sex hormones of the oestrogenic/progestational type were the cause of these malformations.

As a consequence of this published report, I and others have recently been compelled to appear as defendants in civil proceedings in the United States federal courts and have in the process been put to a great deal of inconvenience and expense.

At the trial, however, Dr Gal's conclusions did not stand up to scrutiny. Although in her original report hormonal pregnancy tests were said to be the only reasonable cause of spina bifida in 19 cases of malformed births, it emerged at the trial that in each of the cases cited there was a more probable cause for the neural tube defects than originally suggested. Readers will note that it has taken 15 years for the falsity of these conclusions to be publicly established.

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Whatever happened to chemistry?

Chemists have done wonders in losing their identity in the rest of science. The US successor to the Westheimer report (1965) would give them a new separate identity.

WHATEVER became of the great disciplines into which science was once organized? Mathematicians of different specialities have for many years been unable to speak to each other. Physics, the legend goes, is so balkanized that it is necessary to know where a graduate has been a student before being able to tell what he can be asked to do. And chemistry? Naturally the old division between the inorganic, organic and physical subdisciplines can only have become more entrenched, and thus more separate, with the passage of time.

This question is prompted by the appearance earlier this month of a new report on the state of chemistry from the US National Academy of Sciences (or, strictly speaking, from the National Research Council, its research arm). The objective seems to have been to produce a document that would be a worthy successor to what is generally known as the Westheimer report, the document emerging from a committee under Frank Westheimer, then a Harvard professor, which was the case in 1965 for more grant-making agencies to pay more attention to chemistry. George C. Pimentel, professor of chemistry at Berkeley, has had the invidious task of welding together this decade's prospectus (*Opportunities in chemistry*, National Academy Press, \$18.50 or \$28.50, paper or hardback).

The most obvious feature of the latest document is its hyperbole, which should be forgiven (but also discounted). "Intellectual ferment" is the key word picked out by the academy's press office. In Vannevar Bush's idiom of *The endless frontier*, the Pimentel committee begins by announcing that, in the intervening 20 years, "chemistry has undergone a virtual revolution" and that "new frontiers lie before us". Curious though it may seem, the committee appears to have overlooked the most striking feature of the tale it has to tell, that the intervening twenty years have seen the near-extinction of the old subdisciplines.

It is a curious and unexpected development. The essence of a chemist is that he should be able to recite the Periodic Table and know which parts of it have which properties. This knowledge, the privileged possession of university teachers and their students as recently as half a century ago, is now more widely shared. People who build lasers have been forced to learn the language; so too have those who would design novel semiconductors,

or ternary alloys that are both magnetic and superconducting. Even the chemists' special knowledge of reactions, the feeling for the difference between a first-order and a second-order reaction, for example, has been partly hijacked by astrophysicists eager to know what happens to elements of the Periodic Table in the spaces between stars.

Another influence working in the same direction has been the development of new techniques. Pimentel is right on that. Indeed, the committee might have made more of the character of the change that there has been since Westheimer. Then, in 1965, when the automatically operated pH meter had become a standard laboratory tool alongside the cathode-ray tube, and when newly developed spectroscopic instruments were being manufactured commercially, people had only begun to dream of chemistry laboratories as the centres of electronic excellence they have now become.

The mystique has nevertheless not quite disappeared. Constructing a machine that will assemble chemical ingredients in some predetermined sequence still requires some knowledge of whether the intermediates will be gaseous, liquid, solid or sludges of ill-defined phase. Elementary education will probably still bear the marks of the old subdisciplines for some years to come. Mercifully, perhaps, some teaching establishments will remain more backward than the leaders in the field, with the result that the supply will not entirely dry up of those who know how to diazotize a simple compound without precipitating common salt, or who can extract a globule of shiny metal from a sample of stannous chloride and promise to extract tin by the tonne from rock.

The most important influence in the disciplinization of chemistry has, however, been that of the theoreticians, a still despised body of people when the Westheimer report was written. While the theoreticians' ambitions have never been obviously constrained by over-modesty, their craft has always been indifferent to atomic number. Indeed, those who have been the chemists' camp-followers began by being preoccupied with hydrogen, the simplest of all atoms, and stayed that way for far too long. Often, in Westheimer's time, it must have seemed as if theoretical chemistry was just another way of thinking up novel ways of introducing arbitrary constants into empirical equations. The

time required for difficult notions to mature had obviously been underestimated. Chemists who doubt that might reflect that it is now possible to calculate the mutual potential energy of three or more polarizable molecules when, in Westheimer's time, the mutual interaction of two was beyond them.

So why not boast that chemistry has become more open, less of a discipline? The difficulty is complicated. Pimentel says in his introduction to his report that it is addressed to "those responsible for guiding science policy in the Congress and the Administration", which is another way of saying that it is an appeal to the moneybags. Indeed, the specific recommendations in *Opportunities in chemistry* consist not so much of promises on which chemistry, described as a "central science that responds to societal needs" (the committee's chosen theme), will eventually deliver, but of recommendations that specific grant-making agencies (the US National Institutes of Health and the US Department of Defense) spend more of their resources on the support of chemistry. One particular argument is that there is a need for a proportion of research grants to be larger than is now customary so as to allow for the purchase and operation of expensive equipment.

There is nothing wrong in this; it would be bad not merely for the United States but for chemistry if the support for serious research in the field should sink, as a proportion of general spending on research, to the level now reached in Britain. But there again, the hyperbole needs watching. The Pimentel report says that the "current federal investment in chemical research is still historically rooted in a funding pattern appropriate to a test-tube and Bunsen burner era . . ." Can that be so? With all those NMR machines now somehow scattered about the chemistry departments of United States universities? In a sense, readers will quickly grasp what the committee is trying to say — that this is an important field in which more spending would bring great rewards. But since the moneybags to whom the report is addressed are precisely those who have provided the NMR (and other) machines now at work, there is a danger that the ears of those whom it is intended to impress will be the first to close. Holding up chemistry as a field that has rid itself of old-fashioned disciplinary barriers would have had a similar effect.

John Maddox

Human settlements

Why did the Polynesians abandon their mystery islands?

from Jared M. Diamond

WHEN Europeans entered the Pacific Ocean in the 16th century, they found Polynesians or other peoples living on most islands except for some small isolated or ecologically impoverished ones. Archaeological evidence subsequently showed that at least 12 of these uninhabited islands had previously been occupied by Polynesians and then abandoned.

Why the inhabitants left these islands has been the subject of much speculation^{1,2} — internecine warfare, similar to the killings that nearly wiped out the colony founded on Pitcairn Island by the mutineers from *HMS Bounty*, water shortage, illness, natural disasters, homesickness or lack of women are possible reasons. A recent paper by David Steadman and Storrs Olson³ identifies another possible contributing factor: extermination of the populations of native birds used for food.

The 'mystery islands of Polynesia' include Pitcairn and Henderson, east of the Tuamotu Archipelago, Norfolk and Raoul, off the east coast of New Zealand, Nihoa and Necker, near Hawaii, Palmerston and Suvarrow in the Cook group and several of the Line Islands.

Steadman and Olson have studied Henderson Island, one of the world's most isolated scraps of land, lying over 100 miles east of Pitcairn Island and more than 3,000 miles from any continent. With an area of only 15 square miles, fissured coral terrain and little fresh water, Henderson is still uninhabited and is often considered to have been almost unaffected by man. It is home to 12 species of breeding seabirds and four endemic species of landbirds: a warbler, dove, parrot and flightless rail. The only other vertebrates are a few introduced species.

In 1971, Sinoto⁴ discovered remains of a former Polynesian settlement with a radiocarbon date of about AD 1200–1500. The settlement evidently survived for some time, because the oldest of the three cultural strata included pearl-shell fish-hooks and stone adzes, made of materials absent on Henderson Island and presumably brought by early settlers, whereas the youngest stratum contained shell tools made of inferior local materials after the imported material had been exhausted. The archaeological deposits contained bones of eight species of seabirds, six of them represented by juveniles that presumably had hatched on the island. At least one, and possibly three, of these species no longer breeds there and one of them, the storm petrel *Nesofregatta fuliginosa*, also disappeared from Mangaia in the Cook Islands after Polynesian settle-

ment. There were also bones of two large pigeons of the genus *Ducula*, one of which would have weighed about 0.4 kg and is similar to *D. aurorae* or *D. pacifica*, species that survive on Tahiti and western Pacific islands, respectively; the other would have weighed about 0.8 kg and resembles *D. galeata*, found in the Marquesas. No bones of the usual Polynesian domesticated animals — pigs, dogs and chickens — were found.

Obtaining food on Henderson would have been difficult for these early Polynesian settlers, just as it is today. The steep coastline and lack of extensive reefs or lagoons limit the catching of fish and marine invertebrates. Domestic animals evidently did not arrive or survive, and growing the usual Polynesian crops such as taro, breadfruit and sweet potato may have been difficult on coral terrain with little soil. The settlers may instead have depended for food on the abundant breeding seabirds and on the large pigeons, which, because they had no previous experience of humans, were undoubtedly tame and easy to kill. When these birds

were eventually exterminated, the settlers may have faced starvation or had to abandon their colony.

The evidence for extermination of birds on Henderson Island adds to the growing body of evidence from Hawaii, New Zealand, Fiji, New Caledonia, the Chatham Islands, the Cook group and the Marquesas. On all these Pacific islands the first arrival of humans, Polynesians or their ancestors, was followed by a wave of extinctions similar to the ones that Europeans caused when they first reached islands of the Atlantic and Indian Oceans, and that followed the Indonesian occupation of Madagascar⁵. The evidence from Henderson Island suggests that at least some of the other mystery 'islands' may have been abandoned as a result of over-hunting the birds used as food; extinct birds are also known as fossils from Norfolk Island⁶. Imprudent hunting is evidently not just a modern problem. □

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Solid state physics

Phonon images in semiconductors

from W.A. Phillips

THE pattern shown in Fig. 1, which could be used as an illustration for a book on catastrophe theory, is an image of high-frequency (700 GHz) sound waves propagating in a single crystal of the semiconductor gallium arsenide. It comes from a recent paper by G. A. Northrop, S. E. Hebboul and J. P. Wolfe (*Phys. Rev. Lett.* **55**, 951; 1985). It is no accident that this pattern is similar to the caustics (bright lines) seen when light is reflected on the surface of a liquid in a cup. Both result from geometrical focusing. In semiconductors this focusing arises because the velocity of sound is not isotropic, which in turn is a result of the anisotropy of the crystalline structure and the interatomic forces. These pictures are therefore a sensitive test of our understanding of interatomic forces in solids.

The diagram is a result of an ingenious experiment. A pulse of laser light incident on one surface of the crystal excites electrons from the valence to the conduction band. On dropping back, the electrons emit phonons, quanta of vibrational energy, bearing the same relation to sound waves as photons bear to electromagnetic

waves. This burst of phonons propagates ballistically through the crystal and is detected at a fixed point on its opposite face a fraction of a microsecond later. By scanning the laser across the surface of the crystal the phonon intensity can be measured as a function of propagation direction. Phonon intensity is converted to brightness on a video display to give a phonon image, so that caustics correspond to directions in which phonons are focused on the detector.

Sound propagation in crystals is much more complicated than in gases because of the effect of the regular structure. The dispersion relation, which describes the relation between angular frequency ω and wavevector q is more complex than for, say, sound waves in a gas, where the phase velocity $v_s = \omega/q$ is independent of ω . Experimentally, inelastic neutron scattering is the most powerful technique for studying dispersion relations — the change in neutron energy giving ω and the change in direction giving q . Complementary theoretical studies in essence consider the solid as a collection of particles held together by springs that represent the

interatomic potentials. Experiment and theory agree that the velocity normally decreases as ω increases (although this is not usually significant below 1,000 GHz) and, more importantly for these experiments, that it varies markedly with propagation direction.

A localized wave disturbance (wave packet) does not travel with the phase velocity v_p if this varies with frequency or direction, but with the group velocity $v_g = \partial\omega/\partial\mathbf{q}$. Perhaps the most common example is long-wavelength surface waves on deep water, where the group velocity is half the phase velocity. If v_p varies with direction the effect is more subtle: the direction of propagation, and hence of energy flow, is not parallel to $\mathbf{q}$. This can be illustrated by reference to Fig. 2a which shows schematically, for a typical semiconductor, a contour of constant frequency as a function of q_x and q_y . The sound velocity is anisotropic, so that the contours are not circles. At any point on this contour the direction of energy flow is perpendicular to the contour, as is seen in Fig. 2b, which shows a small section of two neighbouring constant frequency contours. Because δq_x is smaller than δq_y , the component of the group velocity along x is larger, and by a simple geometric argument the resulting group velocity $\mathbf{v}_g$ can be seen to be perpendicular to the contour.

Phonon focusing is a direct consequence of this result. The initial phonon pulse contains a complete range of directions for $\mathbf{q}$, but energy propagation is concentrated in certain directions. For example, at point A on the contour in Fig. 2a the energy contained in a range θ of $\mathbf{q}$ orientations is dispersed into a larger angle θ' , while at B all the energy in a range θ propagates in a single direction. A very greatly enhanced energy flow therefore occurs for directions in which

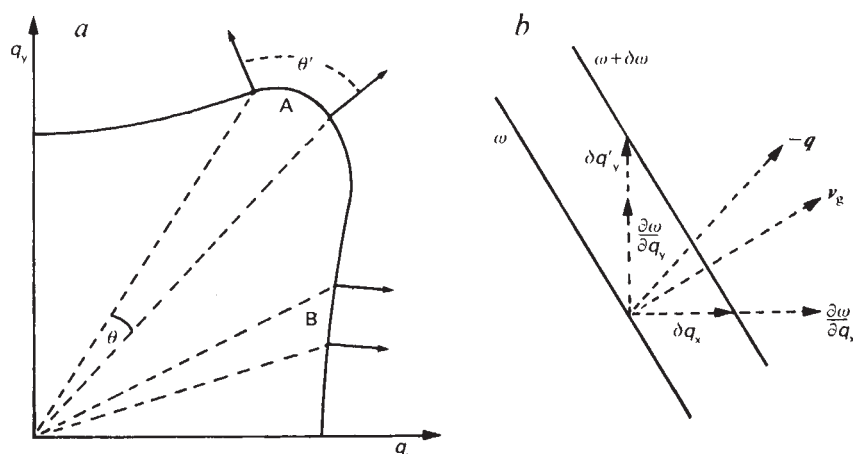


Fig. 2 a, A section through the constant frequency surface for a typical semiconductor in the q_x plane. b, Two closely spaced contours are used to show geometrically why the group velocity is perpendicular to the contour.

the curvature of the constant ω surface vanishes, and it is these singularities that give rise to the caustics in Fig. 1.

Experimental determination of these singular directions provides a more severe test of the accuracy of constant frequency surfaces than does neutron scattering. An even more searching test is the variation of the surface topology with ω , observed as a change in the pattern when higher frequency phonons are selected by the detec-

tor. It is also a testing experiment, since it requires thin crystals to ensure that sufficient phonons reach the detectors, with very small detectors and well-focused laser beams to define directions. Progress will be rewarded by an even more detailed knowledge of interatomic forces in solids. □

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Molecular evolution

Origins of repeated DNA

from John Rogers

DISPERSED within the genomes of all mammals are short DNA sequences that occur many times over. Several families of such dispersed repeated sequences have been identified; how they evolved and what, if any, function they serve has been the subject of much discussion. Until recently, all that has been clear about the origin of these repeated sequences is that two families — *Alu* of primates and B1 of rodents — have evolved from the gene for an RNA molecule (7SL RNA) that performs an essential function in protein synthesis^{1,2}. Recent papers, including one published on page 819 of this issue³, show that several other families of repeated sequences are descended from transfer RNA (tRNA) genes.

Most dispersed repeated sequences in mammals have lengths of 80–300 nucleotides and each occurs about 10^5 times per genome, as exemplified by the *Alu* family in primates. It is thought likely that the repeats are created by reverse transcription of RNAs into DNAs, which are then integrated into the genome; such dispersed copies of RNAs have been designated retroposons (reviewed in ref. 4). The high number of *Alu*-like retroposons is believed to depend on the fact that many individual members of these families can be transcribed by RNA polymerase III (pol III), the promoter for which consists

of sequences within the transcribed gene; therefore, reverse transcripts of many retroposons will retain *pol III* promoter sequences and will be able to undergo further rounds of transcription and reverse transcription. In this way, the sequence can spread through the genome as a form of selfish DNA. This theory of the origin of *Alu*-like families constitutes a null hypothesis which renders unnecessary the numerous and poorly-supported suggestions for *Alu* functions. Although the creation of new retroposon copies has not yet been observed directly, the theory is consistent with the evolutionary lability of such families. They are entirely different in different mammalian orders and can show major differences even between related genera. For example, the so-called ID sequence⁵ is a major *Alu*-like retroposon called R.dre.1 (ref. 6) that has been found near many other rat genes^{4,7} but not, so far, near any mouse genes. So one must ask where these proliferative retroposon families come from.

The first *Alu*-like family to be identified as descended from a tRNA^{4,8} is the C family of artiodactyls (cows and goats). This family has a consensus sequence that is 65 per cent homologous with several tRNAs and can, in theory, be folded up into a secondary structure with all the invariant residues and the four stems and

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Fig. 1 A photoexcited phonon image obtained in a 2 mm thick GaAs (110) oriented crystal. The two bright diamond structures correspond to phonons propagating along $\langle 100 \rangle$ (photo courtesy of J. P. Wolfe).

loops in the normal positions for tRNAs, except for part of the aminoacyl stem. The 'anticodon' of the folded structure corresponds to cysteine; unfortunately, no mammalian tRNA for cysteine has yet been sequenced for comparison.

Daniels and Deininger³ now present two more such examples. One is an *Alu*-like family from the bushbaby, *Galago*, whose consensus sequence is shown to be 68 per cent homologous with the tRNA for the methionine that initiates protein sequences (tRNA^{Met}). Again, this sequence can be folded into a tRNA structure with all the conserved residues in place, so these retroposons must be derived from initiator tRNA^{Met}. The other example is the rat *R.dre.1* family. Although this has no good anticodon or aminoacyl stems, it has perfect D and Ψ stems, and 73 per cent homology to tRNA^{Ala} from an insect (mammalian tRNA^{Ala} has not been sequenced). Even if the obligatory promoter sequences are excluded from comparison, the section between them still shows a sequence match (21/31 with no gaps) that has a probability of less than one per cent of occurring by chance.

For a tRNA sequence to become a proliferative retroposon, it apparently needs to acquire the means of priming reverse transcription from its 3' end. The primer is not known, but all retroposons end in oligo(A) or a similar repetitive structure, and the *Galago* 'monomer' and rat *R.dre.1* both end with oligo(A) immediately after the tRNA-like sequence. The *Galago* sequence is also widespread as part of a heterodimer with the 3' half of the primate *Alu* sequence³, so it is possible that a chance recombination between these two retroposon families has combined favoured elements of each. Accordingly, the consensus sequence of the heterodimer version is further from tRNA^{Met} than is the monomer version³. In the same way, the artiodactyl C sequence has always been found as the 5' element in a heterodimer with a sequence from another retroposon family⁷.

Other major *Alu*-like families — B2 in rodents and C in rabbits (no relation to C in artiodactyls) — have also been screened for tRNA homology^{3,8} but their relationships are more obscure. The B2 family is clearly evolving rapidly; the consensus sequence is substantially different in mice and hamsters, and several major variants are found in mice⁴. Like *R.dre.1*, B2 and rabbit C do not have complete tRNA-like structures and show only moderate similarity to mammalian tRNAs — 60–64 per cent homology, even if the promoter boxes and several gaps are included. (Higher values⁹ arbitrarily exclude the poorly-matching extremities.) B2 and *R.dre.1* show as much similarity to each other^{4,10} as to a tandemly repeated *pol III* gene called *OAX* in frogs¹¹. But the striking homology of *R.dre.1* to tRNA^{Ala} seems to rule out a common origin for all these sequences. This leaves two possible

theories. One is that each of these *Alu*-like families has independently evolved from a tRNA, and their mutual similarities merely reflect the slight homologies between tRNAs. A lack of strong homology to any particular tRNA may either be because the ancestral tRNA has not yet been sequenced, or because the retroposon families have evolved too far. The alternative theory is that the similarities reflect convergent evolution, under selection not only for the promoter sequences but also for additional tRNA-like features surrounding them, in particular the D and Ψ stems. Admittedly, it now seems unlikely that the D and Ψ stems play a role either in the promoter^{4,12} or, in the case of *R.dre.1*, in the secondary structure of the complete RNA⁴; but perhaps they are recognized by reverse transcriptase, especially because tRNAs are the primers for reverse transcription in retroviruses. So, although some retroposon families may have arisen from as yet unidentified *pol III* genes and undergone convergent evolution, a more economical explanation, at present, is that they are descended from tRNA.

Similar arguments apply to some other *pol III* genes. A distant tRNA origin has been suggested for the translation-stimulating *pol III* genes in some DNA viruses¹³; the same suggestion is now made for newly discovered, highly repeated *pol III* genes in lower vertebrates¹⁴. The *OAX* genes in *Xenopus*, also called satellite I, are one such gene family, with more than 10⁴ copies in tandem repeats^{11,15}. The newly defined *pol III* genes from newt and tortoise¹⁴ are even more highly re-

peated, and their genomic organization has not been reported. They are probably not retroposons, as the specimens sequenced have neither the oligo (A) tail nor the terminal duplications that distinguish retroposons, and retroposons are generally rare outside mammals. All three sequences have D and Ψ stems associated with their promoter boxes, and the newt and tortoise sequences show a respectable 69–70 per cent homology to certain tRNAs¹⁴. This strongly suggests that they too arose from tRNAs. But their biological role is unknown; they too await a verdict of useful or selfish, bearing in mind that even if a repeat family has arisen in a useless form, some of its members may still acquire useful roles. □

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Archaeology

Origins of Italian hilltop towns

from Richard Hodges

HILLTOP TOWNS are a striking feature of the Italian landscape. Their origins have attracted considerable attention in recent years since Pierre Toubert's study of village formation in the Sabine Hills¹. Toubert drew upon the voluminous archives of the early mediaeval abbey at Farfa to show that villages within its territory were first settled on hilltops in the tenth century. Village formation (*incastellamento* in Italian) appears to have resulted from the economic explosion in the decades before the end of the Dark Ages around AD 1000. Not all archaeologists and historians, however, have been convinced by Toubert's model. Some believe that nucleated hilltop settlements in Italy date from the collapse of the Roman Empire; others claim that the pattern of early mediaeval settlement was regionally varied, with hilltop villages occurring in some but not all areas from the tenth century. Recent archaeological research throws light on this intriguing debate.

Extensive field surveys in many parts of

Italy conclusively demonstrate that the classical settlement pattern disappeared between the early sixth and early seventh centuries. Towns contracted to mere shadows of their former grandeur², villas and cottages seem to have been deserted in great numbers. Excavations by the British School at Rome have been aimed at finding out what happened to the population — did it mysteriously disappear or were new settlements founded? At Ponte Nepesino, a hilltop site a few miles north of Rome, a small settlement occupied between the sixth and fifteenth centuries has been located. As its name implies, the site overlooks a bridge and may have been settled for its strategic value³. Unlike the stone villas and cottages recently abandoned in the countryside around the site, the early mediaeval dwellings on Ponte Nepesino were of timber and placed haphazardly along the edge of the hill. A palisade enclosed the small community, which resembled a prehistoric hillfort. The standard of living was modest until

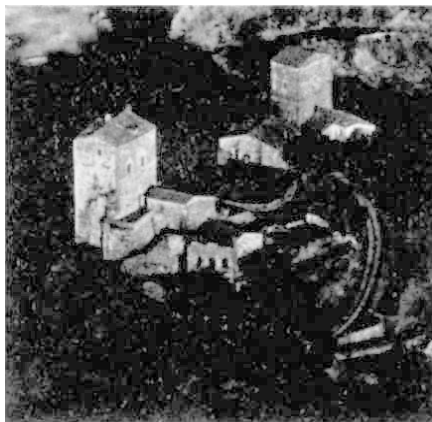
the later tenth or eleventh centuries, when a tower house and accompanying stone-built dwellings were constructed. Associated with these buildings are other evidence of the economic explosion: a variety of mass-produced pottery, glassware and metalwork.

Two similar sites have been recently discovered further south in the region of Molise. Santa Maria in Civita, like Ponte Nepesino, overlooked a Roman bridge; it was also fortified by a palisade with at least two clusters of timber dwellings built up against these defences¹. By contrast, the hilltop settlement of Vacchereccia, close to the early mediaeval monastery at San Vincenzo al Volturno, appears to have been undefended. This settlement was called Baccaricia in the tenth-century charter issued to its inhabitants by the monastery, but recent excavations show that the hill had been occupied since the sixth century. The impoverished pottery from the early phase of occupation mirrors the neolithic; however, Vacchereccia commanded good resources and the limited faunal evidence reveals that domestic production was not entirely primitive. As at Ponte Nepesino, the material culture altered drastically in the tenth century as the San Vincenzo monastery apparently attempted to regulate life in the village more efficiently².

These three excavations suggest not only a dramatic break between the classical and mediaeval worlds, but also between town and country. Rural villas like that recently excavated at Altavilla Salerno³, south of Naples, were abandoned, even though several were then used for other purposes. British School excavations at Anguillara, for example, have demonstrated that a later Roman villa about 40 kilometres north of Rome was turned into a church in the ninth or tenth centuries⁴. Not far away, a later Roman villa lies beneath Pope Hadrian I's monastic farm, dating from the late eighth century, at Santa Cornelia⁵. Likewise it is clear that the Benedictine monks who founded San Vincenzo al Volturno (in Molise) actually refurbished a pre-existing fifth-century villa. The ruins were patched and altered, just as many historians have predicted for monasteries in this period⁶.

Still there is little doubt that there was a dramatic collapse of the rural population in the last years of the Roman Empire. Comparatively few *castelli* of the tenth or eleventh centuries were founded, like Ponte Nepesino or Vacchereccia, in the sixth century; and very few villas were occupied after the demise of the classical state. Instead, as was made clear at a recent conference on *incastellamento*, most hilltop towns and villages originated in the late ninth or tenth century, as Toubert claimed¹⁰.

Scarlino, on the Tuscan coast and recently excavated, is typical¹¹. It sits on a hill overlooking the Mediterranean; the site had been intermittently occupied in



Deserted mediaeval village of Montarrenti, near Sienna, Italy.

prehistoric times, but there are no conclusive traces of early mediaeval settlement. The first village was founded in the tenth century with timber structures around the edge of the hilltop inside a fortification. The pattern resembles that of Ponte Nepesino, but the population was evidently much larger. Like Ponte Nepesino, Scarlino was soon redeveloped. A large castle was constructed in the twelfth century and the village soon became a town sprawling, as it does today, below the imposing citadel. The great wealth of late mediaeval artefacts and rubbish connote a high standard of living, just as do the finds from small excavations within the heart of Orvieto and Sienna. High above their surrounding territories, these hilltop towns seem to have flourished after the turn of the millennium almost despite the limitations imposed by their locations.

The archaeology, therefore, indicates that *incastellamento* in regions like the

Sabine Hills, where Toubert was working, reflect the need for increased administrative control over landed resources (hence, perhaps the increasing number of land charters from this period), as well as a rapid increase in the number of villages. These recent excavations also emphasize the enormity of the collapse of classical civilization, with settlements outliving the demise of the Roman state. Sites such as Ponte Nepesino and Vacchereccia demonstrate the need for defensive sites, possibly commanding territories suitable for practising a simple domestic economy. Such places served as a model for rural settlement as political integration and economic expansion paved the way for mediaeval society from the tenth century onwards. And, as much as anything, they symbolize the break with the classical past and the emergence of mediaeval culture. □

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Earth sciences

Melt migration in the Earth

from David J. Stevenson

MOST of the phenomena studied by geologists and geochemists are due, at least in part, to the ability of fluids to migrate within the Earth's interior. Three processes are believed to operate in melt migration: diapirism (large buoyant masses of partially molten rock, rising by viscously deforming the surrounding solid rock), magmafracturing (the rapid migration of melt along macroscopic fissures in cold rock) and two-phase flow in porous media (the microscopic percolation of buoyant melt in a partially molten rock, and an essential precursor to the other two processes). As a recent paper by McKenzie¹ shows, it is in understanding the third of these processes that the most significant recent developments have occurred.

Flow in porous media has had a quantitative description, known as Darcy's law for over one hundred years. This law, which relates the flow of fluid to the per-

meability of the medium, the pressure gradient driving the flow and the viscosity of the fluid, has been used extensively in studies on melt migration in the Earth's mantle². There are two problems in the application of Darcy's law in its simple form: enormous uncertainties in the permeability, and an incomplete prescription for the pressure gradient driving the flow.

Although problems remain, one major uncertainty in the permeability may have been removed, since it is increasingly apparent from both theoretical and experimental considerations that the melt forms an interconnected network down to arbitrarily small melt fraction, at least for materials which are of geological interest and have achieved textural equilibrium. McKenzie provides strong support for earlier speculations that very low melt fractions (perhaps as low as 0.1%) of low viscosity (water-rich or carbonatitic) magmas can be readily mobilized, with the

interesting geochemical consequences discussed below.

Perhaps more significant, at least from a physics standpoint, has been the development of a 'generalized' Darcy's law which provides a prescription for the pressure gradient by incorporating a constitutive law for the crystalline framework. The problem is this: how does the solid medium allow the withdrawal of magma? Since nature abhors a vacuum, the pores must close. On a geological timescale, this will occur by viscous creep and can be characterized by a bulk viscosity (although the relevant microscopic processes are presumably the same ones that determine the shear viscosity). The buoyancy-derived gravitational power driving the percolative flow is then balanced by two forms of viscous dissipation: that caused by the flow of magma in the microscopic tubules between grains, and that caused by distension or compaction of the solid matrix. If the latter becomes comparable to, or greater than, the former, then the usual simple form of Darcy's law is invalid. This occurs for systems in which the melt fraction varies on a length-scale less than, or comparable to, the 'compaction length' $\sim (\mu_s k / \mu_l)^{1/2}$, where k is the permeability (dimensions of area), μ_s is the appropriate solid-state viscosity and μ_l is the viscosity of the liquid. This is a macroscopic lengthscale of potential geological interest, and can be 100 m–1 km, or even larger. Its most striking manifestation is in the spontaneous formation of solitary waves, discovered during computer experiments^{3,4}.

In one dimension, these shape-preserving waves are horizontal sheets of enhanced partial melt fraction which propagate vertically, riding over a low partial melt fraction. The governing equation is not any of the famous ones analyzed in non-linear wave theory (for example, Korteweg–de Vries) and the waves are probably not exactly solitons in the strict mathematical sense. Nevertheless, these waves have remarkable preservation and are almost exactly conserved after collision with other waves. Most significantly, perhaps, they are readily produced and there is little reason to doubt that they can occur naturally in the Earth. An analogue laboratory system, consisting of waves excited on a fluid conduit and under study at Johns Hopkins University, Woods Hole and California Institute of Technology, exhibits remarkably similar phenomena.

In two dimensions, computer simulations indicate that the one-dimensional waves are spontaneously unstable⁵. Horizontal cylinders or spheres of enhanced partial melt are formed instead and they propagate vertically as well-preserved solitary waves. These 'magmons' probably only occur in regions of slow mantle upflow (and may therefore be unimportant beneath mid-ocean ridges) but could influence the morphology, episodicity and geochemical signatures of a variety of

igneous processes. A convincing demonstration of their existence and importance in the Earth remains a tantalizing goal; the best clues may be geochemical.

Indeed, McKenzie's recent work has focused primarily on the geochemical aspects of our improved understanding of melt migration. In particular, he stresses that as long as one concentrates on major element chemistry, there is agreement between the predicted extent of melting beneath mid-ocean ridges and that inferred from depleted peridotites. Only if one looks at incompatible trace elements is there disagreement. His explanation (similar to that recently put forward by O'Hara) involves the extraction of a small degree of partial melt from a large volume of mantle, which is possible if the first melt has low viscosity and is highly mobile. Since the geochemical and isotopic signature of a differentiation event can depend on the degree of melting, McKenzie also suggests that arguments currently used to estimate the ages of continental crust-forming events require re-examination.

T-cell receptor

The present state of recognition

from Miranda Robertson

The long pursuit of the genes encoding the antigen receptor of T lymphocytes ended last year with the isolation of three genes — the expected α and β genes and a third gene, γ , which was entirely unexpected. Now that more is known about these and other related genes, how much more do we understand about the mechanism of antigen recognition by T cells and the molecular basis of cellular immunity?

MANY will have hoped¹, or feared, that the isolation last year of the genes encoding the T-cell receptor for antigen^{2,4} would quickly dispel much of the controversy and confusion surrounding the molecular basis of cellular immunity. Yet the astonishing progress of the past year or so, which has seen the isolation of seven genes with known or suspected importance in the ontogeny and function of T lymphocytes, has done almost as much to inflame the controversy as to clear the confusion. The controversy is focused chiefly on the question of how many receptor molecules mediate the recognition of antigen by T cells and most of the confusion concerns the definition of functional T-cell subsets. What follows is an account of the present state of the attempt to fit the unambiguous facts of molecular biology into their more equivocal cellular context, partly as reflected at a recent meeting in Aspen*.

The problem of recognition

The central feature of antigen recognition by T lymphocytes is that T cells will recognize foreign antigen only when it is associated on the surface of a cell with a molecule encoded by the major histocompatibility

complex (MHC). This phenomenon is known as MHC restriction and is the single clear operational distinction between recognition by T cells and by B cells (which can see foreign antigen on its own). It also means that the question of antigen recognition is bound up with the definition of functional T-cell subsets, for the following reason.

By functional criteria, there are three major subsets of T cells: the cytotoxic cells that destroy virus-infected cells; and two regulatory subsets, comprising helper and suppressor cells, that modulate the activities of the effector cells, including the B lymphocytes that produce antibodies. The functional definition of suppressor cells is somewhat problematic, and because it now seems that many of them do not express T-cell receptor genes (see ref. 1), the ensuing discussion will be confined to the cytotoxic and helper subsets.

In general, the helper and cytotoxic subsets recognize different classes of MHC molecule: cytotoxic cells usually see antigen in association with class I MHC molecules, which are expressed on all cells (which makes sense because any cell may be infected with a virus); and helper cells usually see antigen in the context of MHC class II molecules, which are expressed largely on the cells of the immune system

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* A conference on T-cell receptors, genetics, structure and function, held at the Given Institute of Pathobiology, Aspen, Colorado, 5–7 July, 1985.

(which makes sense because regulatory T cells need focus only on other cells of the immune system). The question, therefore, is how are the different recognition requirements of B cells and T cells, and of the different T-cell subsets, reflected in the structure of their receptors?

One possibility, raised at the time MHC restriction was discovered, is that foreign antigen and MHC molecules are recognized by separate receptors, and that the receptor for MHC is different in cytotoxic and helper cells. The accumulated evidence of cell biology⁶ and biochemistry⁶ since then has, however, favoured the alternative possibility, namely dual recognition by a single receptor that has been biochemically defined as a heterodimer consisting of one α and one β chain⁶. Moreover, the isolation of the genes encoding the α and β chains has shown that, although they are closely related to those encoding the immunoglobulin molecules that mediate antigen recognition by B cells, they are also distinctively different in ways that could reflect the demands of dual recognition. Fig. 1 illustrates our present knowledge of the genomic organization of the antigen-receptor gene family, including the γ gene which is clearly a member of the family although it is not certain that it encodes an antigen receptor (see later).

Like the immunoglobulin genes, the genes encoding the T-cell receptor are assembled from separate segments, one of which encodes an invariant carboxy-terminal domain known as the constant (C) region, while two or three other segments, known as the V, D and J segments, encode the variable (V) region of the molecule that recognizes antigen. Within the variable region are three regions of hypervariability that form the antigen-binding pocket; it is in the gene segments encoding this region, and particularly in the distribution of the hypervariability, therefore, that significant differences between immunoglobulin and T-cell receptor genes might be expected. Although it is now clear that differences do exist, both in genomic organization and in the pattern of sequence diversity of the V segments that encode the major portion of the variable region, there is no consensus on what it is these differences signify.

Among the distinctive features of the T-cell receptor V genes, the single most prominent is the concentration of diversity at the third hypervariable region, which is the site of the V-J or V-D-J junction (for details see legend to Fig. 1). This point is dramatically illustrated by the most recent data on the α -chain gene pool, described in four recent papers in *Nature*⁷⁻¹⁰. While the immunoglobulin J segments are organized in modest clusters of five or six, the α -chain J segments run to at least 20 in mouse^{8,9} and probably a similar number in man¹⁰, widely dispersed over a region of the genome whose full extent is still unknown.

Is this really important, or is it just an accident of evolution in a generally volatile and duplication-prone gene family? The accident theory leaves rather a lot to be explained away. The β -chain gene pool, for example, also allows for unusual variability in the third hypervariable region, partly through the exceptional scope it offers for combinatorial diversity (for details, see legend to Fig. 1).

The other major distinction between the variable-region coding segments of immunoglobulin and T-cell receptor genes lies in the pattern of germline diversity of the V segments. It is broadly agreed¹²⁻¹⁴ that the V_{β} segments are relatively few (perhaps 20) in number and usually unique, whereas immunoglobulin V segments may run to families 30–40 strong; and that the overall variability of the V_{β} segments is significantly greater than that of immunoglobulin V regions. It is also agreed on the basis of standard techniques for predicting secondary structure that the β chains of the T-cell receptor fold in the same way as the immunoglobulin chains, though a minor squabble has arisen between Hood's laboratory¹³ and Davis's¹² on the justification for the interesting suggestion of Patten *et al.*¹² that the additional variability of the V_{β} sequences may provide extra hypervariable binding sites, possibly for MHC molecules, on the exterior surface of the folded V region.

Now that similar analyses have become

possible for the α -chain genes, it is clear that this distinctive pattern of diversity applies only to the β -chain V segments: those of the α chain are much more like immunoglobulins both in the pattern of their diversity and in their family structure, comprising about 10 subfamilies of some 10 members each^{7,15}. Thus, notwithstanding some dispute^{13,15} on the exact distribution and extent of V_{β} variability, it is fair to say¹⁵ that the α and β V regions seem to have been subject to different selective pressures. This would conform nicely with the idea that the α -chain variable region is adapted to provide a binding site for MHC molecules; but disappointingly, there is not a shred of evidence correlating particular β -chain V regions with differential recognition, either of different classes of MHC molecule or of different polymorphic variants of these two highly polymorphic classes of glycoprotein. On the other hand, nobody seems to have a better idea about the significance of the differences between immunoglobulin and T-cell receptor genes (for instance, why the concentration on hypervariable region three?), and more sequences seem unlikely to help to clarify the problem.

What we need, as Mark Davis pointed out in Aspen, is direct data on antigen binding by the different chains of the T-cell receptor: it is an interesting reflection on the inferential character of the research that led to the isolation of the genes that

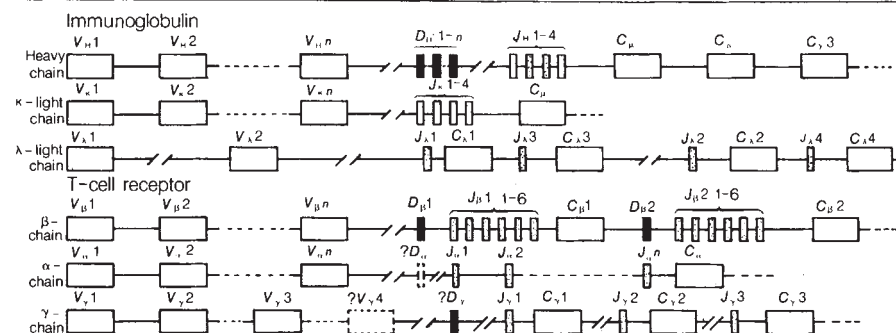


Fig. 1 Organization of the immunoglobulin and T-cell receptor gene families. This scheme is based largely on mouse: the γ -gene pool of man is larger (see text), as is the λ light-chain immunoglobulin-gene pool. The common feature of all six gene pools is that they contain separate gene segments encoding the variable and constant regions of the antigen receptors of lymphocytes (presumptively in the case of the γ gene; see text). In the course of lymphocyte ontogeny, one of the V segments is juxtaposed by chromosomal rearrangement with one of the J segments (and where applicable a D segment) to form a complete variable-region gene. Thus different variable regions are generated by combinatorial diversity. Each V segment has two regions of hypervariability which are known in the case of the immunoglobulins to contribute to the antigen-binding site in the folded molecule. A third hypervariable region, which also contributes to the antigen-binding site, is generated by the junction of the V segment with the J or the D and J segments(s) at recombination. The variety of ways in which the segments are organized and the differences in the extent to which they are duplicated in the different gene pools reflects an eventful evolutionary history under selective pressures that are relatively little understood. It is, however, striking that there is more germline diversity in the T-cell receptor than in the immunoglobulin-gene pools in the J segments. Moreover, in the case of the β -gene pool they are so organized that they also allow more combinatorial diversity. The β -gene pool contains fewer J regions than the α -gene pool, though there are twice as many as in any of the immunoglobulin pools, but it has two distinctive features that allow for exceptional diversification during somatic rearrangement. First, the 'rules' for recombination (see refs 1 and 11) allow in principle the joining of both D to D segments and V segments directly to J segments, neither of which is possible in the immunoglobulin heavy-chain pool although it has both D and J segments. This versatility has not, however, been demonstrated in practice. What has been shown is that the D segments of the β genes can be read in any of the three possible reading frames, so that varying the site of the V-D junction alone could make a substantial contribution to the diversity of the third hypervariable region.

there is so far no direct evidence that the T-cell receptor binds anything at all. Davis is accordingly in the process of developing an expression vector for the production of milligram quantities of the receptor proteins for use in binding studies — and in the longer term for crystallography, which is slower than sequencing but should eventually spoil the fun by providing unambiguous evidence on who binds what and with which and to whom.

Good theories and bad facts

It is partly the failure of the α - and β -chain genes to provide an obvious basis for dual recognition of foreign antigen and MHC that has stimulated the revival of two-receptor theories of antigen recognition by T cells. But perhaps a more powerful stimulus has been the surprise discovery last year in Susumu Tonegawa's laboratory at Massachusetts Institute of Technology of a third gene, also closely related to immunoglobulin, also assembled by genomic rearrangement, and now known as the γ gene¹⁶.

The γ gene has been from the beginning a relentless tease. It was at first mistaken for the α -chain gene, which was in fact isolated a few months later¹⁴. In the meantime, it had already become clear that the absence of the expected potential glycosylation sites and the limited diversity of the γ -gene made that identification unlikely. Further genomic analysis has not provided much clarification. The mouse γ pool is now known to contain three *J* and *C* segments, only one of each of which seems ever to be transcribed in mature T cells¹⁷, and it was at first thought to contain only one functional *V* segment. More recent evidence suggests, however, that there may be four functional *V* segments in mouse (D. Raulet and S. Tonegawa, personal communication) and there are certainly between 6 and 12 in man^{18,20}, though it is not known for either species how many are actually expressed. Indeed, strictly speaking it is not known whether the gene is ever expressed as protein at all: no γ -gene product has yet been conclusively identified (hence the careful avoidance of all mention of γ chains).

Even assuming that the newly discovered *V* segments are expressed, germline diversity remains very limited and the only source of somatic diversification is some variation in the site of the *V*-*J* join. (T-cell receptor genes, including γ and unlike immunoglobulin genes, do not seem to undergo somatic point mutation.) Given the concentration of diversity-generating mechanisms at the *V*-*J* join in the α - and β -chain genes, this may be significant; but even so it is difficult to imagine that the γ -gene repertoire could be large enough to play a significant part in antigen recognition.

For other clues to the functional significance of this unlooked-for gene, Tonegawa has turned to the pattern of its expression during the differentiation of T

Ontogeny of natural killing

IF THE analysis of α - and β -chain gene expression has failed to cast any light on the distinctive requirements of antigen recognition by helper and cytotoxic T-cell subsets, it has succeeded in casting considerable doubt on the authenticity of specific antigen recognition by natural killer (NK) cells. The NK cells, though they do bear some distinctive surface markers of their own, are defined chiefly by default: they will kill a wide variety of target cells without prior immunization or specific MHC associations, and they lack most of the surface markers of the T-cell lineage. Their claim on serious attention lies in their having been implicated in immune surveillance against cancer, though the statistics on cancer mortality are scarcely a testament to their efficacy. Some have held that they are a subset of T cells, others that they belong instead to the macrophage-monocyte lineage.

A careful analysis of T-cell receptor gene expression in parallel with physiological studies on NK cells has now confirmed their membership of the T-cell

lineage but seriously damaged their credibility as a legitimate T-cell subset.

The normal course of T-cell ontogeny is illustrated in Fig. 2, which shows the changes in the expression of a series of molecules first known merely as cell-surface markers and now gradually assuming functions. The first to be expressed is T11, which helps to define stage I thymocytes and has recently been implicated²¹ in the triggering of early proliferation and the induction of receptors for interleukin 2, the growth factor that mediates the further expansion of the thymocyte population. Stage II is marked by the expression of T4 and T8 together, and the rearrangement and transcriptional activation of the T-cell receptor β genes. With further maturation, the cells lose either T4 or T8 and the α -chain genes are activated. This leads for the first time to the expression of the T-cell receptor heterodimer on the cell surface in association with the T3 complex. The mature stage III T cells can now be activated by the binding of antigen to the receptor-T3 complex.

How do NK cells fit into this picture? Nearly all of them, like T-cells, express T11; unlike T-cells, they do not express T4 and most of them do not express T8 or T3. Ritz *et al.* have separately examined T3-positive and T3-negative cell lines and find that only the former express receptor heterodimers on their surface²². (This is consistent with the recent evidence that T3 cannot be expressed without the receptor chains, nor the receptor without T3^{21,22,26}.) The receptor-positive cells, however, represent only something like 5 per cent of NK cells: in the rest, although the β -chain genes are rearranged and transcribed²⁴, they give rise only to truncated RNA that cannot participate in the formation of a functional receptor. This discovery lethally challenges earlier claims²³, based solely on gene rearrangement, for the participation of T-cell receptors in the recognition of antigen by NK cells.

Nonspecific cytotoxicity can, however, be induced in cytotoxic T cells and in NK cells by antibodies directed against T11²⁵. Assuming that the T3-positive 'NK' cells can be regarded effectively as T cells that happen to have an unusual specificity, these data add up to a demonstration that the bulk of NK cells share their early activation and cytolytic mechanisms, but not their recognition mechanisms, with T cells. (Nor do they transcribe the α gene²³). Possibly many of what are known as NK cells may be T cells with functional effector mechanisms but whose recognition mechanisms never grew up.

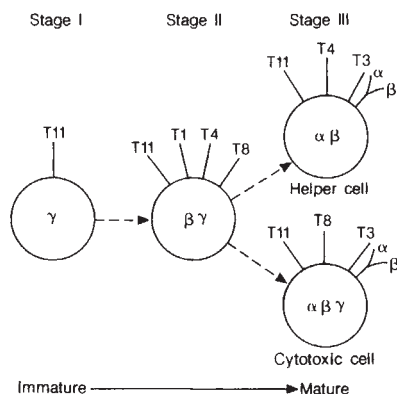


Fig. 2 Ontogeny of T lymphocytes showing cell-surface markers and transcriptional activation of T-cell receptor genes and the α gene. The symbols inside the cells indicate transcriptional activation of α , β and γ genes. The arrows between the stages of maturation are drawn with dotted lines because none of these paths of differentiation is yet formally documented. A further possibility, not illustrated, is that stage I cells differentiate directly into stage III cells. NK cells express the stage I surface phenotype; whether they represent a separate sub-lineage of stage I cells is not certain. For simplicity, T4 is shown on the helper cell and T8 on the cytotoxic cell, though in very rare cases T4 may be expressed on cytotoxic cells or T8 on helpers. In these cases, the cytotoxic cells recognize class II MHC molecules instead of class I and the helpers recognize class I instead of class II. Thus, strictly speaking, the expression of T4 and T8 is associated with differential recognition of MHC and not always with a particular lymphocyte subset.

lymphocytes in the thymus. The γ gene is rearranged and transcribed very early in thymic ontogeny, before the β -chain gene²¹ and well before the α -chain gene, which is the last to be rearranged^{21,23}. After T-cell

maturation, γ -gene transcripts are found only in cytotoxic T cells and at very low levels^{21,24}. This pattern of expression of the two genes has inspired two new two-receptor (or anyway more-than-one-

receptor) theories of antigen recognition by T cells, one published by Pernis and Axel²⁵ and the other by Raulet *et al.*²¹ and elaborated by Tonegawa at Aspen.

Both rest on the premise that the γ gene plays an important part in the recognition of class I MHC molecules by cytotoxic T lymphocytes, and attempt to explain self tolerance (mature T lymphocytes fail to respond to cells bearing 'self' MHC molecules on their own) as well as MHC restriction (they will attack and kill cells bearing a combination of self MHC and foreign antigen). Tonegawa proposes that the γ chain is associated early in ontogeny with the β chain to give binding to self MHC, inducing cell proliferation and thus selection for self reactivity; later in ontogeny he envisages the γ - β dimer giving way to an α - β dimer with a much weaker affinity for self MHC, so that effective binding can occur only with the addition of antigen.

The Pernis-Axel theory is an ingenious effort at explaining not only self tolerance and MHC restriction but alloreactivity as well (T cells will recognize and kill cells bearing non-self MHC). They posit a tetramer composed of two β chains each associated with either a γ chain, to produce self recognition, or an α chain, to produce an antigen binding site; a rarer and more doubtful association of the γ and α chains is supposed to underlie alloreactivity in what, by imaginative use of arithmetic, the authors describe as a one-and-a-half-receptor theory of T-cell recognition.

An untested prediction of both theories is the existence of a fourth gene expressed only in T-helper cells and acting as the equivalent of the γ gene in the recognition of class II MHC molecules. But how well does either theory stand up to the available evidence on the molecular basis of antigen recognition by T cells? The phenomena the two theories were devised to explain are among the most important and problematic in cellular immunology, and the γ gene is undoubtedly the outstanding puzzle of molecular immunology, so it is worth pausing to consider whether each can really help resolve the other.

It remains debatable, for example, whether the limited diversity of the γ gene would be sufficient to allow the binding of the entire range of class I MHC molecules, which are the most polymorphic known to protein chemistry. The expression of γ transcripts at very high levels early in the ontogeny of the thymus is very striking, and a γ - β heterodimer as proposed by Tonegawa is not ruled out, though such evidence as there is suggests that β chains do not emerge at the cell surface until relatively late, when they are coordinately expressed with α chains and the universal T-cell marker T3^{22,23,26}, which is now known to be essential for the activation of T cells by antigen binding (see Fig. 2, in box).

There is, however, compelling evidence against the Pernis-Axel theory, which requires an association between γ and β chains for antigen recognition on mature

T cells. Yagüe *et al.* in the Kappler-Marrack laboratory have investigated α and β gene-loss mutants of three independent T hybridomas, all recognizing the same combination of foreign antigen and MHC. The V segments, and probably the J segments, of both the α and the β genes of the three hybridomas are identical. The loss of either the α or β gene results in loss of recognition; fusion of an α loss mutant with a β loss mutant restores recognition. Thus the α and β chains of the heterodimer seem to be both necessary and sufficient for dual recognition of MHC and foreign antigen.

If the γ gene does not encode a part of the antigen receptor — and assuming that it is in fact expressed on the cell surface — what part might it be playing in the ontogeny of T cells? The prevailing view at Aspen was that the answer probably lies in a consideration of the much broader context of T-cell surface molecules that share their ancestry with immunoglobulin. The full extent of this family of molecules¹¹, whose growth has been charted at intervals in *Nature* by Alan Williams²⁷, has only recently begun to emerge from the sequences of genes encoding the surface molecules originally defined by monoclonal antibodies as differentiation markers of T cells; and it is especially relevant that two in particular, T8 (or CD8)²⁸ and T4 (CD4)²⁹, are now also known to belong to it. These two molecules are expressed respectively on lymphocytes recognizing class I and class II MHC molecules and are strongly implicated in precisely the kind of role imagined for the putative γ chain. Antibodies against T8 block cytotoxic responses, and those against T4 block T-cell help; accordingly it has been suggested that they contribute to the binding of antigen by T cells by recognizing the non-polymorphic determinant on the class I and class II MHC molecules. Some such role as an accessory recognition molecule is generally deemed plausible for the γ -gene product (assuming, again, that there is one). This would, however, make the γ chain the only known non-antigen-receptor molecule to be encoded by a gene that undergoes somatic rearrangement.

Moreover, it is unsettling that such molecular biology as has been brought to bear on the issue of accessory recognition molecules so far has succeeded in undermining somewhat the attractive schema I have just outlined. Golding *et al.* have transfected cells with a class II gene from which the exons encoding the non-

polymorphic domains have been removed. If the binding of these domains by T8 reinforces the specific binding of the polymorphic determinants by the T-cell receptor, then this operation might be expected to abrogate specific recognition. But it does not: alloreactive T cells raised against cells bearing the intact class II molecule will still recognize the truncated one on the transfected cells. This could merely mean that T8 is not always necessary for recognition and binding; however Golding *et al.* have also shown that recognition of such cells is blocked by antibodies to T8, and this is very hard to explain if it is the conserved non-polymorphic determinants of the class II molecules that are involved in accessory recognition. On the other hand, the importance of T8 is clearly affirmed by these experiments and it remains possible that the interaction is with non-polymorphic portions of the polymorphic domains.

Thus while the immunoglobulin family of lymphocyte surface molecules has clearly evolved for specialized interactions in cell-cell recognition, the molecular characterization of its various branches has so far failed to reveal exactly what those interactions are.

It is progress of a sort to learn how recognition of antigen by T cells is not mediated; next year perhaps the availability of cloned genes for all the known and putative recognition structures and the technology for transfecting cells with them will begin to offer some insight into how it is mediated. □

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Miranda Robertson is Biology Editor of *Nature*.

Corrigendum

In the article "Bridging the junctional gap" by Harry Goodall (*Nature*, 26 September, page 286), it was implied that the gap junction studies of N. B. Gilula and A. E. Warner used antibodies raised against junctional membrane preparations. In fact, their studies were conducted with antibodies raised against a 27K protein that was eluted electrophoretically from such preparations.

Stefan Marinov and "friends"

SIR—The article¹ "Stefan Marinov wins friends" oversimplifies the circumstances in which it would be possible to test the possibility of weak failures of Einstein's special theory of relativity. We wish to call attention to a few conceptual points and to clear up the relationship between our paper and those of Marinov, whose experiments we do not endorse.

In our paper², besides the absolute coordinates in the preferred system and Einstein coordinates (this is not relativity!) in the moving system, we also used the co-moving coordinates in the so-called 'new system', which differ from Einstein's coordinates by a Lorentz transformation of the absolute coordinates.

I would like to make these points:

- We postulate (it does not "emerge") that rotating bodies in the moving frame remain rigid in the new system, thus they are not rigid, either in Einstein's co-moving coordinates or in the preferred coordinate frame (postulate A).

- The isotropy of the velocity of light is sacrificed in the new system but is preserved in Einstein's coordinates (used throughout in our paper because of our conviction that most, if not all, of physics is Lorentz invariant). The anisotropy of light in the new system is a coordinate effect, and coordinates are only labels, not "physics".

- We postulate that the angular velocities of the points of freely rotating bodies, at rest in the moving frame, are constant in the new system, so that they are not constant either in the preferred system or in Einstein's coordinates (postulate B).

- We do not propose tests of the modified transformations (the "new system") but of postulates A and B.

On the Marinov issue, I wish to state in a friendly spirit that my interest in possible weak violations of special relativity was aroused at the beginning of 1980 by the analysis of Marinov's controversial papers. The ideas which lead to Marinov's experiments do not conflict with previous experimental results. He described a possible violation of special relativity that seems not to have been considered earlier, that a rotating rod with a transitional velocity V along its axis may not be twisted around the axis when viewed from the absolute system (or from the new system), contrary to the predictions of special relativity. Then it should be counter-twisted when viewed from Einstein's frame.

Marinov was thus implicitly postulating that the rotating axle provides a non-einsteinian clock synchronization procedure which is found to give the absolute time in the new system. Einstein synchronization, using a light pulse, gives Einstein time. Then, contrary to Marinov (who wanted to overthrow special relativity) it

seemed to me appropriate in 1980 that one way of introducing weak violations of special relativity, and of providing for non-relativistic synchronizations, would be to assume postulates A and B, in which case the best coordinate system to use would be Einstein's.

Although Marinov claims that his experiments confirm his postulate, I am convinced that, when repeated independently, they will not reproduce his results. There are a number of contradictions in his two papers. Thus the "coupled mirrors experiment" (to which we referred, not the coupled shutters) is in contradiction with the Fresnel-Newton ether theory which Marinov claims to be using. I shall analyse these experiments in a forthcoming paper and propose modifications in them so that they can really test Marinov's postulate.

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1. Maddox, J. *Nature* **316**, 209 (1985).

2. Maciel, A. K. & Tiomno, J. *Phys. Rev. Lett.* **55**, 143 (1985).

Another novel effect in classical electrostatics

SIR—Recent correspondence in these pages has been concerned with an unexpected result for the minimum energy configuration of a system of equal point charges placed inside a circle. A distinct, but in some ways related, novel result is concerned with the existence of a bound system of positive and negative charges. Thus, consider a single positive point charge fixed at the centre of a circle and equal negative point charges (in general, different in magnitude from that of the positive charge) constrained to remain in the region outside the circle. Negative charges will be attracted by the positive charge to the circumference of the circle and if there are N such charges their mutual repulsion will ensure that they are equally spaced around the circumference. In this case, what is the maximum number of negative charges that can be electronically bound to the circumference by the single positive charge at the centre; that is, what is the number of negative charges that 'saturate' the attractive effect of the positive charge, and what will be the net charge of such a 'saturated' system? Intuitively one might suppose the number required to 'saturate' is the minimum number to render the net charge on the system non-positive. However, we shall see that in general this is not so.

We suppose the negative charges each to have magnitude q times that of the positive charge, and simple energy considerations then show that the maximum value of q for given N is

$$q_m(N) = [g(N) - g(N-1)]^{-1}$$

where $g(N) = (N/4) \sum_{p=1}^{N-1} \operatorname{cosec}(p\pi/N)$.

If we consider the situation where the maximum number of bound negative charges is N , then the maximum and minimum net charge on such a 'saturated' system for varying q will be, respectively, $X_{\max}$ and $X_{\min}$ times the positive charge, where

$$X_{\max}(N) = 1 - Nq_m(N+1),$$

$$X_{\min}(N) = 1 - Nq_m(N)$$

These results are illustrated by the following table.

N	$q_m(N)$	$X_{\min}(N)$	$X_{\max}(N)$
1	∞	∞	-1.000
2	2.000	-3.000	-0.623
3	0.812	-1.435	-0.431
4	0.477	-0.908	-0.310
5	0.327	-0.637	-0.225
10	0.115	-0.149	-0.005
11	0.100	-0.105	+0.021
12	0.089	-0.068	+0.043
13	0.080	-0.037	+0.063
14	0.072	-0.009	+0.081
15	0.066	+0.015	+0.097

Thus for a given value of q there will be a maximum value $N_m(q)$ corresponding to 'saturation'. For $q \gg 1$, $N_m(q) = 1$ and $X_m(q) = 1 - qN_m(q)$ is negative. As q decreases, X_m remains negative with $|X_m|$ decreasing until q passes through the value 2, when $|X_m|$ increases discontinuously from 1 to 3 corresponding to N_m changing to 2 at $q = 2$. For further decrease in q , X_m remains negative with monotonically decreasing magnitude until this magnitude undergoes a further discontinuous increase at $q \approx 0.812$ as N_m changes to 3. This pattern is then repeated continually as q decreases further. However, when q has decreased to $1/11$, $N_m = 11$ and X_m then becomes zero before becoming positive for further decrease in q , followed by it becoming negative again as N_m increases to 12. X_m then oscillates between negative and positive values, becoming zero for $q = 1/12$, $1/13$ and $1/14$ (with corresponding $N_m = 12, 13, 14$). For $q < 1/14$, X_m remains positive, tending to the value of $+1$ as $q \rightarrow 0$ and $N_m \rightarrow \infty$.

We conclude therefore that in general 'saturation' does not correspond to the intuitive idea mentioned at the end of the first paragraph. In particular, the only 'saturated' systems which are electrically neutral are those for which $q = 1/N_m$ with $N_m = 11, 12, 13, 14$. For larger values of q the 'saturated' system always has a net negative charge, while for smaller values of q it is always positive. One wonders whether these ideas have application to any real physical system.

I should like to express my thanks to Mr D.R. Simpson for assistance with numerical aspects of the problem.

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Matter of the fourth estate

Henryk Eisenberg

Polymers: The Origins and Growth of a Science. By Herbert Morawetz.
Wiley: 1985. Pp.306. \$47.50, £48.60.

TO THE three well-known states of matter — gaseous, liquid and solid — we may add a fourth, the polymeric, plastic or macromolecular state. This is well exemplified by a toy-shop item known to all, bouncing putty, a silicone resin with unusual properties. Left to itself on a smooth surface, it slowly flows like a high-viscosity liquid. Shaped into a sphere, in the palms of one's hands, it bounces like rubber. Quickly stressed, it breaks like a solid. It thus incorporates unusual features, dependent on the scale of time. In the book under review, Herbert Morawetz refers to the phenomenon first reported by J. Gough in 1805, that stretching a rubber band releases heat, rather than absorbing it, as would be expected from the usual materials stabilized by strong interatomic and intermolecular forces. We now know that this effect is due to the alignment of macromolecules in the rubber upon stretching, the heat released deriving from the entropy decrease following reduction in the number of rubber chain configurations.

Thirty years ago, excitement among polymer scientists was at a high, as is well documented in Morawetz's book and in some recent recollections of Walter Stockmayer and Bruno Zimm (*A. Rev. phys. Chem.* 35, 1–21; 1984). At that time "Geheimrat" Herman Mark, an active nonagenarian today, often came to our laboratory to demonstrate the latest practical and theoretical advances. A particularly striking example, relating the macromolecular to the macroscopic world, is demonstrated when a non-crosslinked polyvinyl alcohol fibre, to which a small one-gram weight is attached, is immersed in a glass cylinder of almost-boiling water. Polyvinyl alcohol is water soluble under these conditions, yet the small weight promotes chain crystallization and prevents dissolution. The instant the weight touches the bottom of the cylinder and the stress is relieved, the fibre magically disappears in solution.

The property by which macromolecules are distinguished is the possession of long flexible backbones, generated by the addition of bond after bond to, for example, a linear string of carbon atoms. Two out of the four carbon valencies remain free to be filled by hydrogen atoms or bulkier side groups. Chain flexibility and an abundance of chain configurations arise from rotation around the carbon-carbon bonds. Backbones and side groups, additionally or alternatively, may contain oxygen, nitrogen, sulphur, silicon or phosphorus

atoms. Molecular configurations may be variable or lead to fixed conformations in space. Secondary, tertiary and quaternary structures may form and be stabilized by ionic or hydrophobic interactions, or by hydrogen bonds. A wealth of structural features emerge to be used by nature or man.

Locomotion is a particular example in which man has followed a different path from nature. Man invented the wheel and all the noisy clanking machinery with which it is associated. Nature primarily uses sliding and silent macromolecular assemblies of changeable configuration to create exquisite mechano-chemical locomotive devices. In such structures we see the properties of the macromolecular state of matter being applied to great advantage.

In addition to their unusual structural and configurational features, macromolecules possess the capacity of encoding information by the sequential arrangement of nucleotide bases (groupings of phosphate-sugar backbones to which one of four purine or pyrimidine bases are attached) along a DNA chain. These polynucleotide chains carry the sum total of the information required for the maintenance and propagation of the biological entity they represent. They are replicated, transcribed into RNA, processed and translated into proteins, which constitute the machinery to run this unique show.

In the nineteenth century there were considerable advances in understanding

the chemistry of small and moderately sized molecules, and of harnessing their properties for practical use. As stressed by Morawetz in the early chapters of his book, the existence of macromolecules was then suspected but not fully recognized. Not until the 1940s was the resistance of the classical chemist overcome, and were macromolecules, as we understand them today, fully accepted. An era of explosive increase in knowledge followed into the 1960s, and led to spectacular advances in the understanding of the genesis, nature, behaviour and technology of large molecules. These are some of the topics covered by Morawetz. The biological aspects of the subject are outlined but not treated in any detail.

It is, however, perhaps too early to write an objective history of such a young science. All the giants who shaped it lived in our time and almost all were known to us personally. Hermann Staudinger successfully fought the battle for the recognition of the macromolecular state. The Svedberg devised the ultracentrifuge to establish the molecular weight of proteins. Wallace Hume Carothers invented nylon following his investigations into the structure of polymers and their formation by polymerization. Werner Kuhn first calculated macromolecular configurations and estimated the properties of solutions. Peter Debye provided a rational basis for the scattering of radiation. Paul Flory, whose recent and unexpected death came barely two months after we celebrated his seventy-fifth birthday this summer, had, by mid-century, provided basic answers or predictions for all aspects of chain configuration, solution properties and polymerization mechanisms (a striking confirmation of the power of his intuition was the experimental demonstration many years later, by neutron scattering from melts containing polymers and their



Polymers in history — Emperor Wilhelm of Germany (left, in the white coat) and his generals watching his car being fitted with the first synthetic rubber tyres in 1912.

deuterated analogues, that the configuration of polymer chains in bulk is undisturbed and Gaussian). Finally, Karl Ziegler and Giulio Natta, in the 1950s, dramatically introduced the new ionic and coordination polymerization procedures which led to novel and stereospecific polymers.

Notwithstanding the broad significance of polymers in both the life sciences and modern technology, and the intellectual challenge posed in the understanding of their structure, behaviour and function, recognition of polymer science as a distinct scientific discipline was only grudgingly accorded. Morawetz's book, which has the authority to be expected from a distinguished practitioner in the field, appears at an appropriate time to set

the record straight. But while it will be of considerable interest to specialists in polymer science (of which there are many), I doubt if this highly condensed account will appeal to a more general reader wishing to find out what is going on at the other end of the corridor, much less in the building across the road.

In an all-too-short epilogue, Morawetz indicates that polymer science is alive and kicking. The theoretical emphasis is shifting to the condensed state, concentrated solutions and melts, and, on the practical side, there is our every-increasing ability to create a host of novel materials in the service of mankind. □

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Lead into LEED

D.P. Woodruff

Surface Crystallography: An Introduction to Low Energy Electron Diffraction. By L.J. Clarke. Wiley:1985. Pp.329. £31.50, \$49.95.

THE investigation of surface crystallography, particularly of semiconductor surfaces and adsorbed molecular species on transition metals, has a key part in surface science (aimed, in these examples, at gaining a better understanding of semiconductor interfaces and of heterogeneous catalysis). Despite its rather general main title, Dr Clarke's book is concerned almost exclusively with only one of the techniques now used in this field, low energy electron diffraction (LEED). The increasingly important role of several other approaches is given only a token airing in the final chapter.

Two previous books have been published on LEED since its development as a quantitative technique in the 1970s, *Low Energy Electron Diffraction* by J.B. Pendry (Academic, 1974) and *Surface Crystallography by LEED* by M.A. Van Hove and S.Y. Tong (Springer-Verlag, 1979). Both of them are very much concerned with theory, and include computer codes for multiple scattering calculations of diffracted intensities versus electron energy for model structures — structure determination in LEED is achieved through comparison of these calculated intensities with experimental results. The present volume includes no such codes (which are now much more readily available) and rather attempts to present a more general introduction to the subject. The author has been involved in both the collection of experimental data and the running of computer codes, a situation more typical now than it was ten years ago when the theory was still developing.

Generally, Dr Clarke does present a balanced view of theory and experiment,

of physical principles and practicalities. The treatment is not always exhaustive and the author's own interests sometimes intrude, but not unreasonably so. The book is not without its defects, however. At least one section of the audience to which it is directed, senior undergraduates, would appreciate a more ordered introduction to LEED in which more space is devoted to discussion of physical principles. Formulae are sometimes quoted without justification when they could be derived or rationalized quite simply. The basic notation for overlayer surface meshes, introduced by Elizabeth Wood in 1964 and in widespread use in surface science, is first used on p.14 but not explained until p.50. Similarly, early references to the notion of a "coherence length" in electron diffraction are only explained much later, and even then the author fails to clarify the essential error implied by the use of this terminology.

One further and especially unfortunate example of lack of attention to detail appears on the first page of the book. The pioneering LEED experiment of Davisson and Germer, which won them the Nobel Prize, was reported in 1927 in a paper where the authors clearly described the way in which they were led to investigate electron diffraction as a result of an accident which occurred while studying electron scattering. The story is a fascinating one, especially for students, and it is a pity that Dr Clarke incorrectly identifies the accident and the route of Davisson and Germer's discovery of the experimental evidence for the wave nature of electrons. He also promises, but fails to provide, a reference to a bibliography of this early work.

Nevertheless the book will prove valuable to both experienced and would-be surface crystallographers, who should read beyond this introduction but will find that it otherwise provides helpful background material. □

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Collaboration in the revolution

Andrew McMichael

Histocompatibility Testing 1984. Edited by E. D. Albert, M. P. Baur and W. R. Mayr. Springer-Verlag: 1985. Pp.764. DM 340, \$98.

THE international histocompatibility workshops, initiated over 20 years ago, are among the best examples of large-scale international scientific collaboration; for instance, it is unlikely that the serological complexity of the highly polymorphic HLA system could have been unravelled by now without such organized co-operation. Until the ninth workshop, reported in this volume, serology ruled (although cellular typing techniques made an important impact at the sixth meeting in 1975), and alloantisera, obtained by natural immunization during pregnancy, have been used in microcytotoxicity assays to define the A,B and C antigens of HLA Class I and the subsets of HLA Class II. This report, however, portends the revolution — monoclonal antibodies, two-dimensional gels and Southern blots feature alongside the customary two-by-two tables and correlation and frequency tables.

A prime function of the book, continuing the role of its predecessors, is to serve as the dictionary of HLA serology. This is achieved in a series of brief and lucid joint reports describing, in turn, each HLA antigen. Cytotoxic monoclonal antibodies now have an important role, and their present and future contribution is clearly assessed in a review by Julia Bodmer.

The pilot collaborative study on the use of two-dimensional gels to analyse HLA Class II antigens, precipitated with monoclonal antibodies, was surprisingly unsuccessful. There was unexpected heterogeneity in the results from different

Autumn books

The Autumn books review supplement will appear in two weeks time, in the 14 November issue of *Nature*.

The books to be reviewed include *The Background of Ecology*, *The Cosmological Distance Ladder*, *The Dialectical Biologist*, *Mathematical People*, *Vaulting Ambition: Sociobiology and the Quest for Human Nature*, *Seven Clues to the Origin of Life*, *The Joy of Science and Chemistry in America, 1876 – 1976*.

Textbooks

Nature's annual textbook review supplement (which in 1985 covered over 100 books for undergraduates and college students) will next year appear on 27 February. Publishers on both sides of the Atlantic are invited to send review copies of texts published during 1985 to the book review editor in the London office.

researchers due to many small differences in technique. The value of the method, however, is demonstrated unequivocally in reports from individual laboratories where it is shown to be capable of identifying the polymorphisms and splitting the Class II antigens into subsets. Useful lessons for the future were learned from the experience and are spelled out in the joint report by M. J. Crumpton *et al.*

Work on cellular typing techniques also demonstrated their value. It is clear that the splits of Class II (and Class I) antigens defined by T cells are real and have a molecular basis, indicated here by individual reports comparing HLA Class II antigens seen with two-dimensional gel analyses and DNA restriction fragment length polymorphisms (RFLP). Although no joint analysis was made of Southern blots, individual reports explored the potential of the technique. At the time of the meeting (early summer 1984) the results were still relatively preliminary, but it is evident that a new dimension has been added to research in this area. These data are eminently suitable for the type of collaborative experiment and analysis at which the workshops excel, and no doubt will feature prominently in the future.

One might ask where this mass of information is leading. Successful organ transplantation was the original aim, and it may become feasible to match genes (and flanking DNA) between donors and recipients. However the extreme polymorphism makes this intention unrealistic in most cases and one will then have to compromise with the best fit. Serological matching of HLA antigens is thus likely to remain the most valuable method of tissue typing. However, matching for transplantation, which is reviewed in detail in this volume, is not the end of HLA. The disease associations remain unexplained and this enigma clearly drives much of the research effort. The search is now on for disease susceptibility genes in linkage disequilibrium with the serologically defined HLA antigens and for disease-specific variants of known HLA antigens. This detailed genetic information should give insight into many, if not all, of the disease associations. Again, this type of study will form a major part of the next HLA workshop. This is now at the planning stage, and it is clear that there will be a greater emphasis on biochemical and DNA technology.

This book is an essential reference volume for all those interested in any aspect of HLA. It is full of detail but also included are helpful summaries in the form of figures, tables and reviews. The editors deserve our thanks not only for organizing this huge experimental undertaking but also for producing such a useful report. □

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Towards a new phase in the cell cycle

Paul Nurse

The Biology of Cell Reproduction. By Renato Baserga. *Harvard University Press: 1985. Pp.251. \$23.50, £19.95.*

LONG recognized as one of the most important areas of cell biology, research into the mitotic cell cycle is entering an exciting new phase. The techniques of molecular biology and genetics are now being used to supplement more classical approaches and are providing real insight into how the cell cycle may be controlled. Renato Baserga's timely book is concerned with this question and with explaining the fundamentals of the cell cycle to molecular biologists, and recent molecular advances to cell biologists.

Baserga has succeeded well in meeting these objectives. The book provides a succinct general introduction to animal cell reproduction and to the possible controlling roles of growth factors, oncogenes and cell cycle genes. It is particularly good when dealing with the effects of viruses on the cell cycle and with the relationship of the cell cycle to cell population and tissue growth, strengths which probably reflect the author's background as a pathologist. The level of treatment of the subject matter is generally very even; little previous knowledge is assumed and the reader is taken to the point where the more specialist literature can be tackled. This, together with the informal, anecdotal style of writing, make the book easy to read

and suitable for advanced students.

The author has decided to restrict the material almost entirely to mammalian cells "to keep the book within reasonable limits". Although this limitation does indeed bring a sharp focus to the book, it does have its drawbacks. Studies of the cell cycle and its control are well advanced in organisms such as the yeasts, *Physarum* and *Xenopus*, and discussion of these could have provided a useful framework for understanding the undoubtedly more complex situation in mammalian cells. I also found the account of the more theoretical and conceptual aspects of the problem to be rather limited. Ideas such as "commitment" or the notion of rate-limiting steps in cell cycle control are not fully considered, nor are the various possibilities for temporal order such as transition probability models, limit cycle oscillators or dependency relations.

Further, surprisingly little attention is given to the two major processes of the cell cycle, S-phase and mitosis. Quite often books on the cell cycle give short shrift to these events, usually referring the reader to a more general textbook. This is an unfortunate practice; when trying to understand overall cell cycle control, it is important to know the nature of the processes which are controlled as well as being conceptually clear as to what the controls are. Baserga does not quite get to grips with these matters, but his book should still be read for its sensible account of the biology of animal cell reproduction and for its up-to-date summary of molecular changes during the cell cycle. □

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Going to work on . . . oogenesis

Tim Hunt

Oogenesis. Developmental Biology: A Comprehensive Synthesis, Vol. 1. Edited by Leon W. Browder. *Plenum: 1985. Pp.632. \$75, £71.25.*

WE ALL started life as eggs, yet the number of books about oogenesis — how the egg comes to be — is very small. To my knowledge, *Oogenesis* is only the third monograph to be devoted exclusively to the subject since 1961.

This is surprising. A tremendous amount of work is done on eggs. However, many more people follow the egg as it develops after fertilization than study its development before fertilization. Not only is the former generally more exciting but oogenesis is really quite difficult to study. Still, the events that occur in early embryogenesis demand a knowledge of what went before. In many cases, the

embryonic axes and parts of the embryo-to-be are already laid down by the time of fertilization. Moreover, eggs viewed simply as cells are extremely interesting. Recombination and meiosis occur in them, and they also exhibit a tantalizing paradox — they are highly specialized for rapid cell division after fertilization, but they cannot (and must not) divide before the sperm arrives. How the solution to this paradox is achieved, and what stops premature cleavage is a very topical concern. Even the simple matter of keeping the embryo alive after the egg has been laid presents physiological problems which in different animals are solved in different and unusual ways.

Thus a new book devoted to the subject is to be welcomed, and warmly so, for it is a well-produced and useful volume. Its 13 chapters cover a wide and fairly representative set of topics, from yolk to shell at the beginning and from genes to proteins at the end. For the most part the organization is phylogenetic: Chapters 1,3 and 5 mainly deal with vertebrates, and 2, 8 and 13 with insects; frogs are the subject of 6, 9 and 10; and sea urchins are discussed in

Chapter 12. There are over 90 pages of references, running up to 1983 which is about the norm given the production schedule for a book of this sort.

The claim to comprehensiveness, expressed in the subtitle of the series, seems to be justified as far as the chosen topics are concerned, but the editor Dr Leon Browder admits that some areas have had to be left out because of the "open-ended nature of the topics". Thus while there is a rather heavy emphasis on yolk and shell — interesting stuff, as it turns out, with an especially good chapter by R. Wallace — there is nothing on egg polarity, on embryonic determinants (except insofar as the last chapter, "Genetics", touches on this issue) or on meiosis, kinetochores or the synaptonemal complex. Given these omissions, it is odd to find a whole chapter devoted to annulate lamellae, one of the last cytoplasmic organelles whose role is still uncertain.

The chapters on the control of gene activity are pretty dry reading, with the stress more on the "comprehensive" in the series title than the "synthesis". The RNA found in oocytes still seems to be poorly understood despite much detailed study; and lampbrush chromosomes are apparently as mysterious as ever. A chapter devoted to ribosomes would have been at least as appropriate as the one on annulate lamellae. There is, however, a very clear account of 5S RNA synthesis in frog oocytes by A. Kramer (though it does seem odd that it wasn't written by D. Brown or Kramer's ex-colleague, R. Roeder). And while J.B. Gordon doesn't actually work on oogenesis as such, he and his colleagues' work on the stage 6 *Xenopus* oocyte has been so copious and influential that the absence of a contribution from him or any of his associates struck me as slightly strange. It is also a pity that several other well-known authorities in the field had already contributed to Vol. 1 of the new edition of Metz and Monroy's *Biology of Fertilization* (Academic Press, 1985).

This collection is more coherent than many other examples of the multi-author genre of book (eggs is eggs, after all), but an introductory overview would have been helpful. The nearest thing is A.W. Schuetz's long opening chapter entitled "Control Mechanisms of Oogenesis and Folliculogenesis" which has some good things in it especially towards the end. But I was lost all too often in this essay, as starfish, mice, pigs, frogs and fish flashed briefly by. Schuetz's opening sentence, which begins the book, is also faintly worrying: "In most animal species a single cell, the female gamete, is the primary or only cellular link between the present and successive generations". Are there really animals that *don't* have eggs? □

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In the past climate

John Birks

Quaternary Paleoclimatology: Methods of Paleoclimatic Reconstruction. By R. S. Bradley. *George Allen & Unwin:1985. Pp.472. Hbk £30, \$50; pbk £13.95, \$24.95.*
Paleoclimate Analysis and Modeling. Edited by Alan D. Hecht. *Wiley:1985. Pp.445. £63.25, \$66.45.*

IAN Ball (*Systematic Zoology* 24, 407; 1975) has suggested that many sciences develop in three stages: from (i) a descriptive phase when patterns are detected and described, through (ii) a narrative phase when inductively based explanations are proposed for the observed patterns, to (iii) an analytical phase, with a hypothetico-deductive basis, in which competing hypotheses about underlying processes are tested. Quaternary palaeoclimatology is in transition from a narrative to an analytical phase, and is thus acquiring what Popper terms "scientific maturity".

In the past 20 years, there have been enormous advances in the subject, ranging across a number of areas of research — analysis of ice-cores, marine sediments, and lake and bog sediments; stable-isotope stratigraphy; tree-ring analysis; studies of lake-level and other geological features; and the compilation of historical and documentary evidence. In his book, Bradley provides readable and critical reviews of all these topics at a level suitable for undergraduates and research students. He also gives an excellent overview of dating techniques for the Quaternary, the basic requirement for any reliable correlation of past climatic events.

The emphasis throughout is on reconstruction and hence on the descriptive and narrative aspects. There is little on the use of palaeoclimatic reconstructions to test hypotheses about causes of climatic

change, for example by providing input parameters for simulations of climatic patterns by global circulation models. Bradley has thus taken a geological rather than a climatological approach, and so discussion of some of the very recent trends in palaeoclimatic research is absent. Indeed the author himself comments that the subject is so huge that "a book purporting to survey the field would have been better written by a team of specialists" (p. ix).

This is exactly what is to be found in *Paleoclimate Analysis and Modeling*, in which Hecht has brought together nine contributions by 14 leading authorities. Here is a truly exciting overview, not only of descriptive and narrative palaeoclimatology but also of the new developments in analytical palaeoclimatology involving computer modelling and rigorous testing of hypotheses about causes of long-term climatic change. The contributions are all excellent, and outline relevant methodologies, available databases, and recent applications and reconstructions. They show with great clarity how biostratigraphy and geological evidence can provide the much-needed historical perspective that allows us to appreciate the magnitude of past climatic events in both time and space, and to understand the mechanisms of long-term changes in the past. The emphasis is very much on current research, particularly in the United States. This is an advanced book, and as such will be an invaluable source for all Quaternary scientists.

We can fully expect that future work in Quaternary palaeoclimatology will be as fruitful as that of recent years. These two books will, in their different ways, stimulate and attract students and researchers to the subject, and hence contribute further to the solution of what John Imbrie and Katherine Imbrie have called "the mystery of the ice ages". □

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Drawing by John Walcott (1754/55–1831) of the rook, together with an outsize representation of the "pernicious grub" on which it feeds (to the overall benefit of farmers, according to Walcott). The illustration is taken from *Bird Etchings: The Illustrators and Their Books, 1655–1855*, by Christine E. Jackson, which includes biographies of sixteen amateur naturalists together with full-page examples of their work in both colour and black-and-white. Publisher is Cornell University Press, price is \$55 in the United States, \$60.50 in Britain.

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REASONS

The secular variation of Earth's magnetic field

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Models of the magnetic field at the core-mantle boundary at selected epochs from 1715.0 to 1980.0 reveal novel features in the field at the core. These suggest that the flow of core fluid is coupled to the mantle, and that magnetic diffusion is significant.

THE Earth's magnetic field varies on timescales ranging from seconds to millions of years. The variations on timescales shorter than ~ 5 yr are dominated by sources of external origin. Variations over longer timescales, which are collectively known as the secular variation (SV), and which are of predominantly internal origin, provide an extremely useful tool for investigating the dynamics of the Earth's fluid outer core. Observations have been made of the Earth's magnetic field for perhaps a thousand years¹; reliable observations date back over 300 yr², and provide a potentially extremely valuable data set.

Two problems have, in the past, prevented full use being made of these data. The methods used to analyse the data have been inadequate because of not taking due account of the problems of using observations made on, or just above, the Earth's surface to model the magnetic field at the core-mantle boundary (CMB) some 3,000 km below. Additionally, previous analyses have been based on secondary collections of data which are very much less reliable and comprehensive than the original sources.

To learn as much as possible about the processes responsible for generating the SV and the field itself it is crucial to have models of the field at the CMB. Features of the SV which have been identified at the Earth's surface may have a quite different expression at the CMB.

The two principal features of the SV, as observed at the Earth's surface, are the westward drift, first recognized by Halley^{3,4} three centuries ago and amounting to around 1° in 5 yr, and the decay of the dipole component of the field by 7% since 1845. The westward drift has been interpreted both as being due to bulk westward motion of fluid in the core relative to the mantle⁵, and to westward-propagating slow magnetohydrodynamic waves in the core⁶, with no mass motion. The decay of the dipole could be due to an intrinsic oscillation of the dynamo process⁷ or alternatively due to the re-arrangement of field lines by fluid motions near the CMB. Recent theoretical work⁸ has suggested that the latter possibility can, under certain assumptions, be discarded. The lack of reliable field models at the CMB covering a sufficiently long time interval has, in the past, precluded the possibility of discriminating between these possibilities.

Other work on the surface expression of SV has involved the separation into drifting and standing parts⁹⁻¹². Separations have been made using the spherical harmonic representation of the SV, rather than on the basis of the identification of particular features at the CMB.

Regional differences in the surface SV have also been observed. Low SV in the Pacific region, together with an appreciably smaller non-dipole ingredient of field than in other regions, was first recognized by Fisk¹³. Palaeomagnetic evidence suggests that these features of the SV in the Pacific region may have persisted over a very much longer timescale¹⁴⁻²³. The lack of models of the field at the CMB has precluded anything other than highly speculative interpretations being made of these features²⁴.

During the past 20 yr attempts have been made to use models of the SV to calculate the fluid velocity at the CMB, with the

simplifying assumption, the Roberts and Scott frozen-flux hypothesis²⁵, that the effects of magnetic diffusion are negligible over the timescales considered and the length scales resolved by the available data. Early attempts²⁶⁻²⁸ paid insufficient attention to the problem of non-uniqueness; Backus²⁹ showed that only the component of core velocity normal to curves of zero radial field (null-flux curves) could be determined uniquely. Others³⁰⁻³³ have shown that it is possible to determine additional components of the flow by making additional assumptions. Backus also derived necessary conditions for the data to satisfy the frozen-flux hypothesis: that the flux through patches on the CMB bounded by null-flux curves should not change with time. These provide powerful tests of the hypothesis.

Magnetic field models

A full description of the compilation of the historical data set (the pre-twentieth century data) is in preparation; the models presented here differ from all models produced by previous workers in that they are based on original magnetic field observations. Previous studies (for example, refs 34-38) have been based on either the catalogue of Veinberg and Shibaev³⁹, which consists of data reduced to regular grid-points at fixed time intervals, or on data obtained from charts of the field. Some of these studies have included archaeomagnetic data in conjunction with the catalogue data, but in no case has the original data itself been analysed. The data set compiled for this study is considerably more extensive than that used by Veinberg and Shibaev.

The twentieth century data set comprised observatory annual means, survey and repeat station data from the 'Main Field' data set supplied by World Digital Data Centre-C1 (Edinburgh), and satellite data supplied by NASA Goddard Space Flight Centre.

Before 1832 there were no observations of the absolute intensity of the geomagnetic field⁴⁰; only measurements of the field direction were made. This poses two problems: first we can, at the very most, determine only the relative intensities of the geomagnetic coefficients; the absolute intensity must be fixed by some other means. We rely on the extrapolation of the decay in the axial dipole term given by Barraclough³⁷. Second is the more fundamental problem of uniqueness: are direction measurements alone sufficient? Notwithstanding the analysis of Kono⁴¹, no acceptable proof or counter-example has been published to resolve this issue. Accordingly, some caution must be exercised in the interpretation of models based on angular data alone, although the obvious similarity of features in these early models to those in the later models suggests that non-uniqueness is not a serious practical problem.

The problem of inferring the magnetic field at the CMB from surface observations is an example of a continuous inverse problem. Unless we introduce additional assumptions into the inversion procedure, we can conclude nothing about the magnetic field at the CMB. In the past, it has been assumed that the field can be adequately represented by a severely truncated spherical harmonic expansion, degree 8 being the favoured choice of truncation level—a wholly unwarranted assumption. Two legitimate approaches can be adopted for introducing additional assumptions, or previous information, into the inver-

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sion procedure: algebraic inverse theory, pioneered by G. E. Backus, F. Gilbert and R. L. Parker and applied to the geomagnetic inverse problem by Shure *et al.*⁴²; and stochastic inverse theory, applied to geophysical inverse problems by Jackson⁴³, and to this problem by us⁴⁴. Stochastic inversion and harmonic splines, as the application of algebraic inverse theory by Shure *et al.* is known, result, in appropriate circumstances, in identical field models; however, the error estimates which can be derived from these two methods differ significantly. Stochastic inversion results in statistical error estimates, that is errors representing the uncertainty in the derived field model, while harmonic splines can only provide absolute bounds on the range of values which can be taken by the models, although such bounds have yet to be derived. Stochastic inversion, with appropriate choice of previous information results in finite point error estimates; harmonic splines results in unbounded point values. Both methods ultimately rely on the validity of the underlying assumptions. Stochastic inversion, which we have used in this study, has the advantage of being considerably easier to implement. Previous information was specified in terms of the second choice of norm, the dissipation norm⁴⁴.

The 1715.0 model is based on 2,575 observations of the declination (including 200 observations made by Halley, and corrected by Barraclough) and 142 observations of the inclination, drawn from the period 1695–1735. The errors assigned to the data were derived from three parts: a component due to crustal fields; a component due to secular variation (none of the observations was corrected for secular variation); and a component due to observational errors. The observations were checked exhaustively for navigational errors; it should be remembered that the determination of longitude at sea was very poor during this period. Many reported longitudes were found to be in substantial error and corrections made.

The 1777.5 model was produced in a very similar fashion using 4,733 declination observations and 1,382 inclination observations, drawn from the period 1765–90, including many observations from the voyages of Captain James Cook. The navigation corrections were less drastic because of the advances in determining the longitude at sea which were introduced from 1770.

The 1842.5 model was derived almost entirely from data compiled by Sir Edward Sabine^{45–49}; from this compilation we used 4,503 observations of the declination, 4,725 of the inclination and 3,170 of the absolute field intensity, covering the period 1830–60. Sabine's corrections for secular variation were applied where available.

The twentieth century models were based on a data set comprising observatory annual means, repeat station, survey and satellite data. Corrections were applied to observatory annual means to remove the influence of crustal magnetization using the method described by Gubbins and Bloxham⁴⁴. The weighting of the data followed the method used for recent field models^{44,50}.

The radial component of the magnetic field at the CMB is plotted for each of these models in Fig. 1. Table 1 gives the estimated error in the radial field at the CMB for each model, together with the model norm (a measure of the roughness of the field at the CMB), and the trace of the resolution matrix (effectively the number of degrees of freedom).

Immediately apparent is the remarkable degree of resolution afforded: small scale features in the field are resolved even in the 1715.0 field model. The fact that models based on entirely different data sets, spanning almost 300 yr, show such a high degree of coherency provides extremely strong evidence that the error estimates given in Table 1 are reasonable, and that most of the small scale features in the core field can be believed. However, it is possible that the stochastic inversion will result in error estimates which are too small if too much reliance is placed on the prior information. There is some evidence of this effect in Table 1: the norm for the 1715 model is very small compared with the other models, because stronger damping has been used to produce a smooth field model; more reliance has been placed on the prior information, to compensate for the poorer data.

Characteristics of secular variation

From the series of models shown in Fig. 1 we can readily identify various features in the field at the CMB.

Rapidly drifting flux spots. These intense patches are observed in the Southern Hemisphere from around 90° east. They drift westwards towards South America, with changes in intensity. We can trace four such spots, labelled A–D in the plot for 1980 in Fig. 1, through many epochs. Some other spots either drift too fast, merge with others, or are too poorly resolved for us to correlate without better time resolution.

Static flux bundles. These are permanent regions of intense flux observed under arctic Canada, Siberia and under Antarctica. For a dipole field we would expect one region of intense flux at each pole, but instead we observe two such regions near, but not at, the North Pole (one under Canada and one under Siberia) and, in most models, two under Antarctica. Static flux bundles are also observed under the central Pacific Ocean and the Persian Gulf. These features have remained virtually stationary for more than 250 yr.

Static zero flux patches. These permanent regions of very low flux are observed at the North Pole, under Easter Island, in the northern Pacific Ocean and, in many models, near the South Pole. Zero Flux is particularly surprising near the poles, which is where a dipole field would have maximum intensity of flux.

Localized field oscillations. Large amplitude oscillations occur in the field under Indonesia in the region of the undulations of the magnetic equator. These oscillations do not propagate despite their wave-like appearance.

This description differs from what has previously been inferred from maps of the surface field. Westward drift occurs only in certain well-defined regions of the core; indeed, in the northern Pacific Ocean there is slow eastward drift. Flux spots are observed to drift rapidly westwards, while flux bundles appear to be static. The separation into drifting and standing features is not possible by wavelength alone: the wavelengths of both categories are similar.

The low SV observed under the central Pacific is directly associated with that at the surface, but the field in this region at the CMB has a significant non-dipole ingredient (the central Pacific flux bundle) while the surface field is predominantly dipolar. The central Pacific flux bundle is of sufficiently short wavelength to have only a relatively small effect on the surface field.

The high SV observed in southern Africa and Indonesia is due to rapidly drifting core spots, and the large field oscillations, respectively.

The succession of relative 'highs and lows' in the field observed under Europe and the north Atlantic Ocean is suggestive of a wave-like behaviour. The models presented here, at intervals of ~60 yr, have inadequate time-resolution to investigate their propagation. A series of models at 10-yr intervals throughout the twentieth century is being used to investigate them.

It is possible that some of the static features may be crustal anomalies which have been mapped into the core field. Core and crustal fields cannot be separated by any direct means. It is frequently argued⁵¹, from examination of the power spectrum

Table 1 Estimated error, model norm and trace resolution matrix for models of the radial field

Field model	Error at CMB (μT)	Model norm ($\times 10^{12}$)	Trace resolution matrix
1715.0	55–125	47	54
1777.5	85–130	88	83
1842.5	70–100	132	102
1905.5	50–70	130	112
1969.5	40	137	155
1980.0	30	136	150

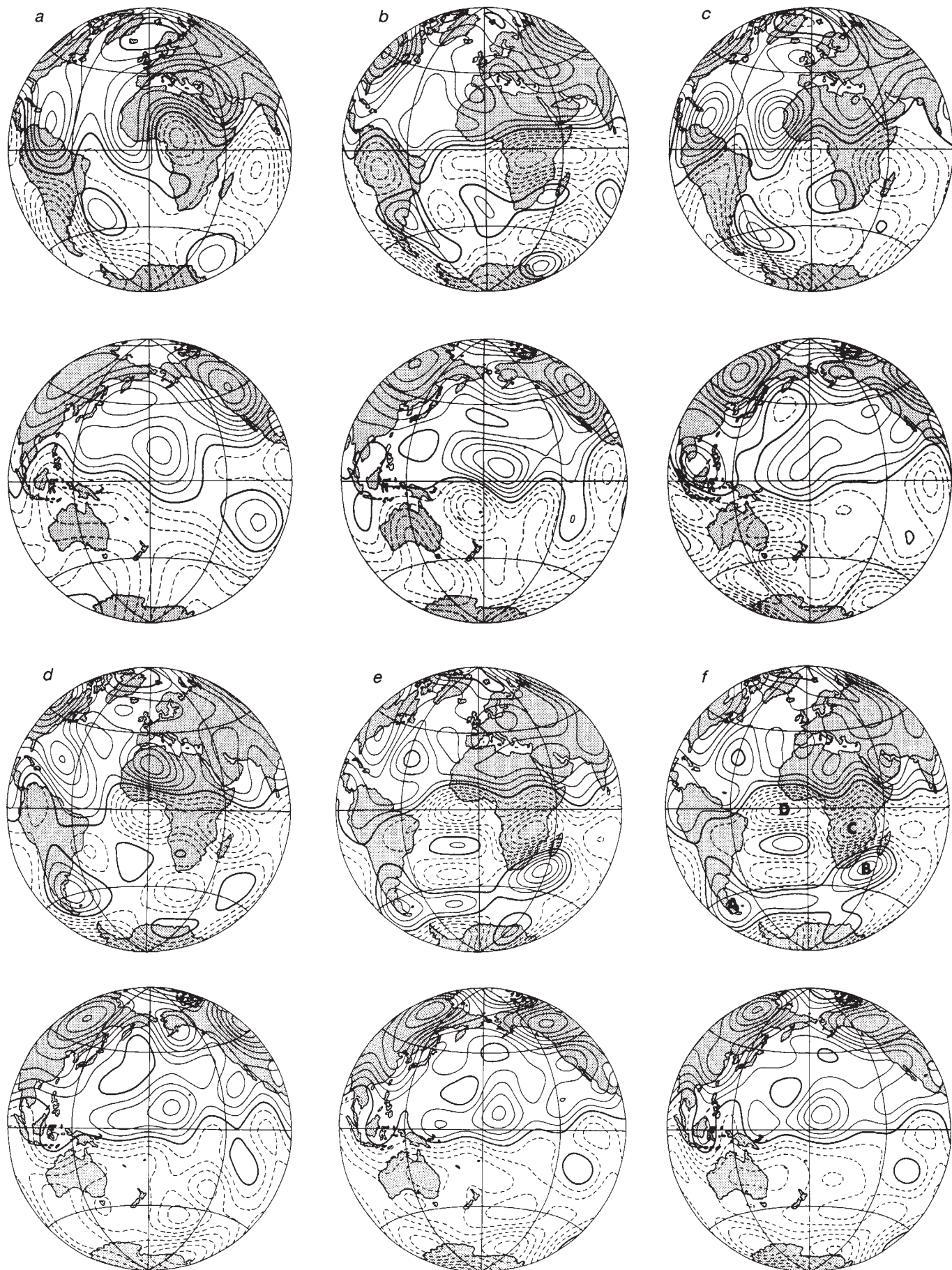


Fig. 1 Contour plots of the radial field at the CMB for: *a*, 1715.0; *b*, 1777.5; *c*, 1842.5; *d*, 1905.5; *e*, 1969.5; and *f*, 1980.0. The contour interval is $100 \mu\text{T}$; solid contours represent flux into the core, broken contours flux out of the core. The bold contours represent zero radial field. The labels A-D in *f* are referred to in the text.

of the total observed field, that the core field dominates up to degree 13 and the crustal field above degree 15. However, such a separation is totally *ad hoc*, and has no justification other than being convenient and appearing reasonable. An alternative approach, which has been developed by Meyer and colleagues^{52,53}, is to tackle the problem by forward modelling. They produce global models of the petrology of the Earth's crust and calculate the induced magnetization. The method reproduces the shape of the observed power spectrum, supporting the interpretation of the power spectrum. Their method only models induced magnetization; over continental areas remanent magnetization varies over very short length scales and so can be ignored at the wavelengths in which we are interested. Longer wavelength components of remanent magnetization may be important over the oceanic crust⁵⁴.

The crustal model CRST-70-F-22-22 (ref. 53) was removed from the 1980.0 data set before analysis, as an initial check for contamination by induced crustal sources: this had no noticeable effect on the core field, tending to confirm that these models are not contaminated by induced sources of crustal magnetization.

Interpretation of secular variation

Our core field models are inconsistent with Backus's necessary conditions on the frozen-flux hypothesis. Patches of reversed flux that are under the south Atlantic Ocean have intensified, in violation of the frozen-flux requirement that flux through patches bounded by null-flux curves be conserved. The integrated flux through these patches is given in Table 2. This result vindicates our earlier tentative conclusion⁵⁰, based on only 20 yr of data, that we had detected diffusion of magnetic field at the CMB. The topology of null-flux curves also changes. The constraint that the topology of null-flux curves be invariant (that they do not merge or divide) is weak because only a very small amount of diffusion is required to violate it.

Rejection of the frozen-flux hypothesis may have fundamental consequences: almost all models of the core flow have been produced from models of the main field and SV (for example, refs 31–34, 55–57) which have been derived assuming the frozen-flux hypothesis; the validity of these models must now be reconsidered. To judge the usefulness of the frozen-flux hypothesis as a first approximation to the kinematics of the secular variation we examine the changes taking place under the South Atlantic region in more detail. Figure 2 shows the flux through the patches in this region for 1905 and 1969. Although the division of the South Atlantic patch into two parts in 1969 is not formally justified, these two parts are only linked by a thin 'neck' (at around 15° east). The intensification of flux has been largely confined to the region beneath southern Africa, through which the flux has increased severalfold during the 69-yr interval, suggesting that the frozen-flux approximation is not a good approximation in this region.

What could cause these large changes in flux? One possible mechanism is the expulsion of toroidal field from the core into the mantle. Such a phenomenon is known to occur on the Sun producing sunspots. Allan and Bullard⁵⁸ examined the effect

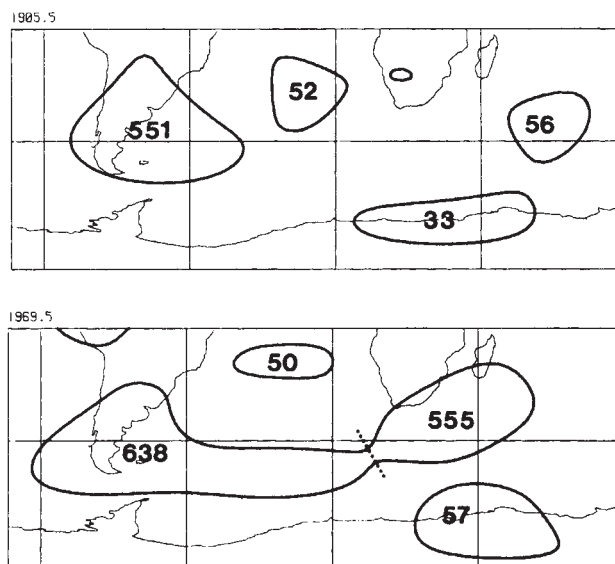


Fig. 2 Flux (in MWb) through reversed flux patches under South Atlantic Ocean region in 1905.5 and 1969.5.

of upwelling on a toroidal field, for low magnetic Reynolds numbers, as did Nagata and Rikitake^{59,60}. We are concerned here with magnetic Reynolds numbers as large as 10^2 and are compelled to investigate the process numerically.

We have investigated, using a simple two-dimensional model, the effect of upwelling on an initially uniform, horizontal field. The field diffuses quite rapidly through the rigid, insulating boundary. For example, with a magnetic Reynolds number of 10^2 , and an initial horizontal field strength of 10 mT, the strength of the vertical field above the boundary increases by up to 1 mT in one quarter of the convective turnover time, say 75 yr. More importantly, the rate of flux expulsion is found, before the approach to steady state conditions, to be relatively insensitive to the value of the magnetic Reynolds number, suggesting that if upwelling motions do exist near the CMB in regions of toroidal field then the validity of the frozen-flux hypothesis is not guaranteed by a large magnetic Reynolds number alone. This process of expulsion of toroidal field into the mantle results in pairs of patches of intense flux of opposite sign, which can intensify rapidly. The rapidly drifting flux spots fit this picture. This process offers an explanation of the rapid changes observed in the flux integrals under the southern Atlantic Ocean, without the need for low core conductivity. The rate of expulsion of flux may be different for the spherical problem.

The dipole has decayed by 7% during the time interval from 1842.5, the earliest model based on intensities, through to 1980.0. During this same period the reverse flux under the south Atlantic Ocean has increased substantially in intensity. These patches of reverse flux are directly related to the decrease in the dipole intensity: adding flux of the 'wrong' sign reduces the dipole moment.

We propose a diffusive model for the static flux bundles. Downwelling of fluid will sweep field lines in towards a point on the CMB, resulting in a region of concentrated flux. In the steady state, the resultant feature will be limited by diffusion, with magnetic flux being swept in by the flow at the same rate as flux diffuses away. Note that the central Pacific flux bundle is surrounded by areas of zero flux to the east, north and south-west suggesting that the flux is being swept out of these regions into the bundle.

To make the suggestion quantitative, consider axisymmetric cylindrical flow in a half space, in which flow at the surface converges towards a point O on the boundary—the intersection of the axis with the surface. Because O is a stagnation point of the flow, we can linearize the velocity components around O and so reduce the induction equation to a simple ordinary

Table 2 Total flux through reverse flux patches in Southern Hemisphere (MWb)

Field model	Flux
1715.0	321
1775.5	520
1842.5	711
1905.5	697
1969.5	1300
1980.0	1362

Totals are for all reverse flux patches in the Southern Hemisphere except the Easter Island patch.

differential equation valid near O. The solution has a gaussian profile

$$e^{-(s/\delta)^2}$$

where s is the distance from O, and δ is the characteristic width of the bundle with

$$\delta^2 = 2\eta L / V\pi$$

and V is a characteristic velocity, L is a characteristic length scale of the convection, and η is the magnetic diffusivity.

The flux in the central Pacific flux bundle is concentrated into a region of characteristic width 1,000 km. Taking 2,500 km as a horizontal length scale for the convection, and $\eta = 2.6 \text{ m}^2 \text{ s}^{-1}$, we find $V \sim 5 \times 10^{-6} \text{ ms}^{-1}$, a value consistent with the very small velocities inferred from the eastward movement of field lines to the north of this region.

Diffusion times of features in the core field are hard to estimate for they depend on the unknown radial field structure. The central Pacific feature corresponds to angular mode 8, which, for a radial mode of three or four, results in a diffusion time of $\sim 400 \text{ yr}$ —sufficiently short for us to have observed diffusive decay. This suggests that the feature is being maintained against diffusive losses by advection of field.

The static patches of zero flux are similarly explained by upwelling of fluid; horizontally diverging flow sweeps away field lines creating a region of very low, or zero, field. This very simple view of upwelling will be complicated by the presence of toroidal field which may be brought to the surface by the flow, although at the poles, where we do not expect a strong toroidal field, this simple view is probably sufficient; indeed, zero flux patches are observed near both poles.

The existence of upwelling at the CMB has an important bearing on studies of the thermodynamics of the core⁶¹. Whaler⁶²⁻⁶⁴ used models of the SV to test the no-upwelling hypothesis, using necessary conditions similar to those used to test the frozen-flux hypothesis. Under the no-upwelling hypothesis, flux should be conserved through patches on the CMB bounded by any contour of radial field, instead of for just zero contours. Whaler found these flux integrals changed less with time than integrals over arbitrary patches on the CMB, supporting the no-upwelling hypothesis. However, the validity of this test rests critically on the validity of the frozen-flux hypothesis. If we relax the frozen-flux constraint then downwelling flow can result in steady-state features, such as static flux

bundles, for which flux through patches bounded by contours of radial field will be conserved. In fact, downwelling flow will ultimately result in small intense regions of flux for which the frozen-flux constraint, and consequently the test for downwelling, will fail.

Discussion

We have shown that the SV is influenced by electrical diffusion in the core. Diffusion will inevitably be significant at some level; its detection depends only on the quality of the available data. We have shown that the historical observations are sufficiently accurate and well-distributed to exhibit the effects of diffusion.

This result may appear to limit the usefulness of the frozen-flux hypothesis in determining core motions. This is not necessarily the case as the frozen-flux hypothesis may still be useful in finding a first approximation to the core velocities. Diffusive effects may then provide additional information on smaller effects such as the slow flows, discussed above, that produce flux bundles and zero flux patches. Other diffusive effects, however, may be quite incompatible with the frozen-flux concept; one such mechanism is the expulsion of toroidal field to produce spots. The resulting SV cannot be modelled using the frozen-flux hypothesis, and any core motions calculated using the hypothesis may be wholly misleading in such regions.

It is unlikely that the pattern of core flow remains stationary with respect to the mantle, so the existence of static features in the core field, which do not appear to be crustal in origin, suggests that the flow in the core may be coupled to the mantle. Three coupling mechanisms are plausible: thermal, electromagnetic and topographic. Temperature variations in the mantle may drive flow in the core, a cold spot in the mantle producing downwelling of fluid (such as beneath the central Pacific) and a hot spot upwelling resulting in a zero flux patch. Alternatively, baroclinic instabilities may be established producing more complicated flow. Electromagnetic coupling could arise from variations in the electrical conductivity of the mantle which would influence the magnetic field, and possibly the core flow through the Lorentz force. The idea of topographic coupling is an old one⁶⁵: bumps on the CMB may influence the core flow. The oscillations in the field beneath Indonesia might be associated with topography. There is recent evidence for larger-scale variations in the seismic velocity at the base of the mantle⁶⁶, which could be due to temperature variations, as well as dynamically-maintained topography at the CMB.

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The three-dimensional structure of *trp* repressor

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The crystal structure of the Escherichia coli trp repressor has been solved to atomic resolution. The dimeric protein has a remarkable subunit interface in which five of each subunit's six helices are interlinked. The binding of L-tryptophan activates the aporepressor indirectly by fixing the orientation of the second helix of the helix-turn-helix motif and by moulding the details of the repressor's structure near the DNA binding surface.

THE synthesis of L-tryptophan in enteric bacteria is regulated in part by repression of *trpEDCBA*, the biosynthetic operon for L-tryptophan, and *aroH*, which encodes one of the isoenzymes that catalyses the first step in aromatic amino-acid biosynthesis^{1,2}. Repression of these operons is regulated by a straightforward negative feedback loop; as the concentration of L-tryptophan increases, two molecules of L-tryptophan bind with a K_D of about 10^{-5} M to the inactive *trp* aporepressor, a dimer of two identical 107 amino-acid subunits³. This reaction forms the active *trp* repressor which binds specifically and firmly as a dimer to the operators of these operons^{3,4}. By the same mechanism, the *trp* repressor is also autoregulatory since it decreases transcription of the *trpR* gene that encodes the *trp* aporepressor polypeptide. The *trp* repressor has been purified, characterized and crystallized in a form suitable for high resolution structure analysis⁵.

The crystal structure determination of three DNA-binding regulatory proteins⁶⁻⁸ has provided a general framework with which to understand protein interactions with regulatory sequences in DNA. The structural basis of *trp* repressor's function is of special interest because its regulatory role is modulated by the binding of a specific small molecule, L-tryptophan. Altered patterns of crystallization⁵ and proteolysis⁹ suggest that binding L-tryptophan produces conformational changes that may underlie the activation of the aporepressor to the repressor. The repressor has also been subjected to intense genetic analysis¹⁰.

We report here the three-dimensional structure of the *Escherichia coli trp* repressor as determined from an electron-density map of the trigonal crystal form at a resolution of 2.6 Å. Many features are clear even in the partially refined model. These are: (1) the unusual architecture of the dimer, (2) the tryptophan binding site and the more obvious aspects of L-tryptophan's role as an activator or co-repressor, (3) the general features of the repressor/operator interaction, and (4) the distribution of mutational changes in the repressor in relation to their effect on DNA interactions and L-tryptophan binding.

Structure determination

Trigonal crystals of *trp* repressor, that is, the active complex between L-tryptophan and aporepressor, were grown as

described in ref. 5 and then changed to a phosphate-free stabilizing solvent containing 2.37 M $(\text{NH}_4)_2\text{SO}_4$, 0.4 M NaCl, 2.4 mM L-tryptophan and 50 mM Na acetate pH 5.4 to facilitate the formation of an especially good uranyl acetate derivative. The space group is $P3_221$ with cell dimensions $a = b = 50.6$ Å and $c = 73.6$ Å. The asymmetric unit contains one-half of the functional dimer; thus, the two subunits are related by exact 2-fold crystallographic symmetry. An electron-density map was calculated to 2.6 Å resolution by the multiple isomorphous replacement (MIR) method using three heavy-atom derivatives: $\text{UO}_2(\text{CH}_3\text{CO}_2)_2$, HgCl_2 and $\text{K}_2\text{Pt}(\text{CN})_4$ (Table 1). Data were collected on film by the oscillation method. Bijvoet differences were measured for the uranyl derivative because of the anticipated strong anomalous scattering signal. This expectation was verified by the fact that the uranium positions could be easily determined from a Patterson synthesis of the Bijvoet differences. These differences contributed significantly to the phase determination (Table 1) and resolved the ambiguity of the space-group enantiomorph. The mean figure of merit for the 3,350 Fourier coefficients used to produce the map was 0.74 across the full resolution range. Restrained least-squares refinement using the Konnerth-Hendrickson program¹¹ is in progress; the current *R*-factor is 27% for all data to 2.6 Å resolution with retention of excellent stereochemistry.

The polypeptide chain was readily traced through an MIR electron density map in which the backbone density was virtually continuous. The molecular model was built and adjusted with the computer graphics programs GRINCH and FRODO¹² implemented on an Evans and Sutherland PS330 hosted by a VAX 11/750. Contrast between solvent and repressor molecule was strong, and side chains were consistently well represented by density except for some of the surface residues. A section of the MIR map with the model superimposed is shown in Fig. 1. The molecular boundary was clearly outlined with the dimer axis located on one of the two lattice dyads.

Dimeric architecture and DNA binding motif

Amino-terminal sequence analysis showed that the crystalline *trp* repressor subunit starts with alanine at position 2 of the 108 amino-acid polypeptide predicted from the gene sequence¹³.

Table 1 Quality of the MIR phases

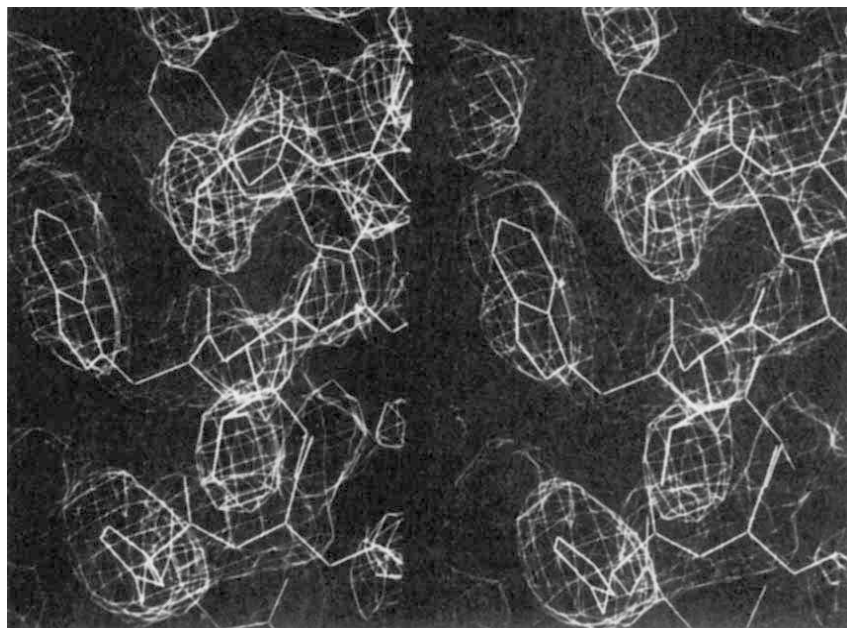
Resolution*	10.8	7.4	5.6	4.5	3.8	3.3	2.9	2.6	Overall
Figure of merit†	0.78	0.85	0.83	0.76	0.74	0.74	0.73	0.70	0.74
Phasing power‡ of:									
Uranyl acetate	3.61	4.16	3.96	3.06	2.94	2.95	3.16	3.00	3.17
(anomalous dispersion)	2.66	1.76	1.78	2.20	2.14	1.82	1.46	1.44	1.73
Mercuric chloride	1.33	2.50	2.62	2.05	1.44	1.19	1.14	0.96	1.61
Platinum cyanide	1.71	1.53	1.63	0.96	0.80	0.99	1.13	1.20	1.08

* Upper limit of resolution shells in ångströms.

† Figure of merit is a measure of the relative reliability of a phase based on the consistency of the MIR analysis. The highest reliability is indicated by the maximum value of 1.0; the minimum value is 0.0.

‡ The phasing power of a derivative is the calculated strength of the heavy-atom contribution (or anomalous dispersion difference) divided by the estimate of the error in the analysis.

Fig. 1 Interpretability of the MIR electron density map. A central segment of the A helix superimposed on the map. The side chains of His 16, Trp 19 and Phe 22 are prominent.



Seventy-seven of the 107 residues (72%) form six helices (A-F) connected by short turns (Fig. 2). All but one of the helices (helix D) of one subunit make extensive contacts with some part of the opposing subunit, making the dyad-related subunits so completely interlinked that it would appear difficult to disengage them without significantly altering their tertiary structures. If the monomer is envisioned by itself (dark subunit in Fig. 2) it lacks the 'globular' shape that is typical of most proteins and their subunits. Helix A and helix B at one end of the subunit are separated from helices D, E and F at the opposing end by nearly the full length of the five-turn helix C. The structure of an individual subunit is reminiscent of the recently reported structures of troponin C^{14,15} and calmodulin¹⁶. Like troponin C, the long helix (here helix C) connecting the globular domains is bent at a central Gly residue (here by 12° at position 52).

The fact that most of the tertiary structural interactions are provided by contacts between the subunits raises questions about the folding process in this molecule. How, for example, would a newly synthesized polypeptide chain fold and preserve itself before dimerization? The unusual structure of the dimer interface and its puzzling implications raise the possibility of a mistaken interpretation. This possibility is ruled out by the fact that except for the terminal segments noted below, the entire MIR electron density map is strong and continuous, and unambiguously matches the side chains in the interpretation presented here (Fig. 1). Any attempt to make a more globular shape for the monomer would require ignoring continuous density with

well-defined side chains between helices B and C and, instead, spanning at least 10 Å of discontinuous density.

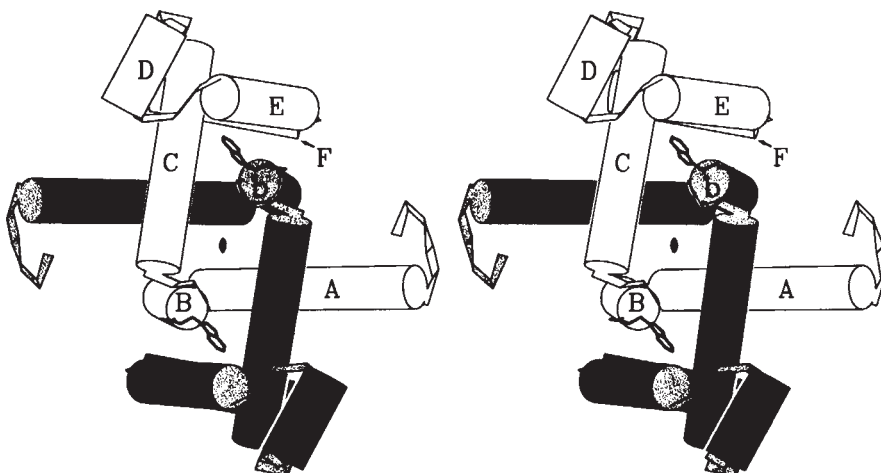
Residues 68-92, containing helix D and helix E, form a helix-turn-helix DNA-binding structural element that has a conformation homologous to the α_2 - α_3 regions found in *cro* and in the amino-terminal fragment of λ repressor¹⁷, and the α_E - α_F structure of catabolite gene activator protein¹⁸ (CAP). The two-turn D helix is somewhat irregular with an enlarged second turn. In their sequence analysis of many site-specific DNA-binding proteins, Ohlendorf *et al.*¹⁹ showed residues 68-88 of *trp* repressor to be homologous in sequence and presumably in structure with those that form the helix-turn-helix motif in *cro*, λ repressor and CAP and predicted that it would have a similar DNA binding function.

The first three and the last two residues of the subunit are not well represented in the current map, indicating that they have a less well-defined structure in the crystal and presumably also in solution. A poorly structured amino-terminal segment has been implicated in several DNA binding regulatory proteins^{20,21}.

Tryptophan binding site

The crystal structure reported here is of the active repressor and contains two equivalent molecules of bound L-tryptophan per dimer⁵. The position of the corepressor ligand was established as the only significant unassigned density after completely fitting the polypeptide. It is strong continuous density, unconnected

Fig. 2 Architecture of the *trp* repressor. A schematic stereo representation, as 'viewed' by the operator down the molecular dyad, showing helices as straight cylinders and connecting turns as folded tape. One subunit is displayed in black. The molecules of L-tryptophan that act as the co-repressor are drawn in skeletal form. The residues (codon sequence numbering) that comprise the helices are as follows: A, 10-31; B, 35-42 (b labels the same helix in opposing subunit); C, 45-63; D, 68-75; E, 79-92; F (obscured in the figure by helices E and C) 94-105.



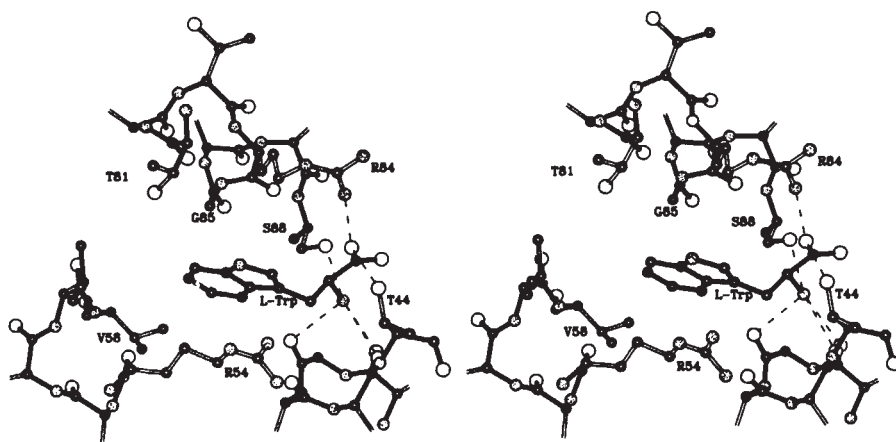


Fig. 3 L-Tryptophan binding site. A stereo pair showing the essential features of L-tryptophan binding in one of the two symmetrical binding sites in the *trp* repressor. Possible hydrogen bonds to L-tryptophan based on acceptable distance measurements (2.8–3.2 Å) are shown by dashed lines.

to the backbone, and it is well modelled by L-tryptophan. On the edge of this density near the 5-position of the indole ring is seen the only significant peak in a difference map (not shown) contrasting repressor containing iodo-L-tryptophan with the parent structure containing underivatized L-tryptophan. This L-tryptophan site (Figs 2–5) is located in a natural cavity near the surface of *trp* repressor formed by the sides of helices C and E of one subunit and the turn between helices B and C of the second subunit; that is, both subunits contribute to each of the two symmetrical tryptophan binding sites.

Figure 3 shows the detailed structure of the L-tryptophan binding site seen at this early stage of refinement. The following points are noteworthy: one surface of the tryptophan site is provided by Gly 85 where the indole ring of the bound tryptophan occupies the space that is usually taken by a bulkier hydrophobic side chain (often tryptophan) at the homologous position of the helix–turn–helix regions in other sequence-specific DNA-binding proteins¹⁹. However, unlike a constituent of the polypeptide chain, the α -carbon substituents of the bound L-tryptophan point away from the main chain atoms of Gly 85. The carboxyl group of the co-repressor interacts directly with the guanidino group of Arg 84 and the hydroxyl of Thr 44 in the opposing subunit. The bound tryptophan's α -ammonium group appears to be hydrogen-bonded to the hydroxyl of Ser 88 and the C-terminal end of the opposing subunit's B helix through the main-chain carbonyls of residues 40 and 41. The hydrophobic pocket for L-tryptophan is formed primarily on one side of its indole ring by the proximal arm of Arg 84 that bends toward and bridges with the carboxyl of the bound tryptophan, and on the other side of the indole ring by the extended aliphatic arm of Arg 54, whose guanidino group forms a hydrogen bond to the backbone carbonyl of residue 42 and a salt bridge with Glu 47, both of the opposing subunit. The direct involvement of the side chains of both Arg 54 and Arg 84 in tryptophan binding may explain the observation⁹ that these sites are more susceptible to trypsin cleavage in the aporepressor than in the repressor. The hydrophobic pocket is completed by Ile 57 and Val 58; no aromatic side chain interact with the bound tryptophan.

Operator binding by model building

A preliminary model for the complex between repressor and operator was made by 'docking' the *trp* repressor structure with a molecular model of the operator using the model building program FRODO. Sequence comparisons and mutational analysis²² indicate that an idealized operator would be a 2-fold rotationally symmetric B-DNA duplex with the sequence shown in Fig. 4c. Thus, when a complex is formed the idealized *trp* operator and the *trp* repressor are likely to share a common 2-fold axis. For these initial studies a symmetrical operator was constructed from the coordinates of a helically uniform B-DNA²³ into which was inserted the 20-base consensus sequence. The proposed sequence-dependent variations in helical param-

eters^{24,25} were not imposed at this stage, nor was the helical axis of the operator bent as suggested for *cro*²⁶ and CAP binding sites²⁷. We chose the interactive surface of the operator to be that side of the DNA which placed the two major grooves at symmetrical positions one turn of the helix apart. In this way the contour of the chosen side makes a distinctly better stereochemical fit with repressor; in particular, the repressor can readily contact the bases that have been implicated as the principal recognition sites by studies of operator constitutive mutants and sequence homology among the related operators²².

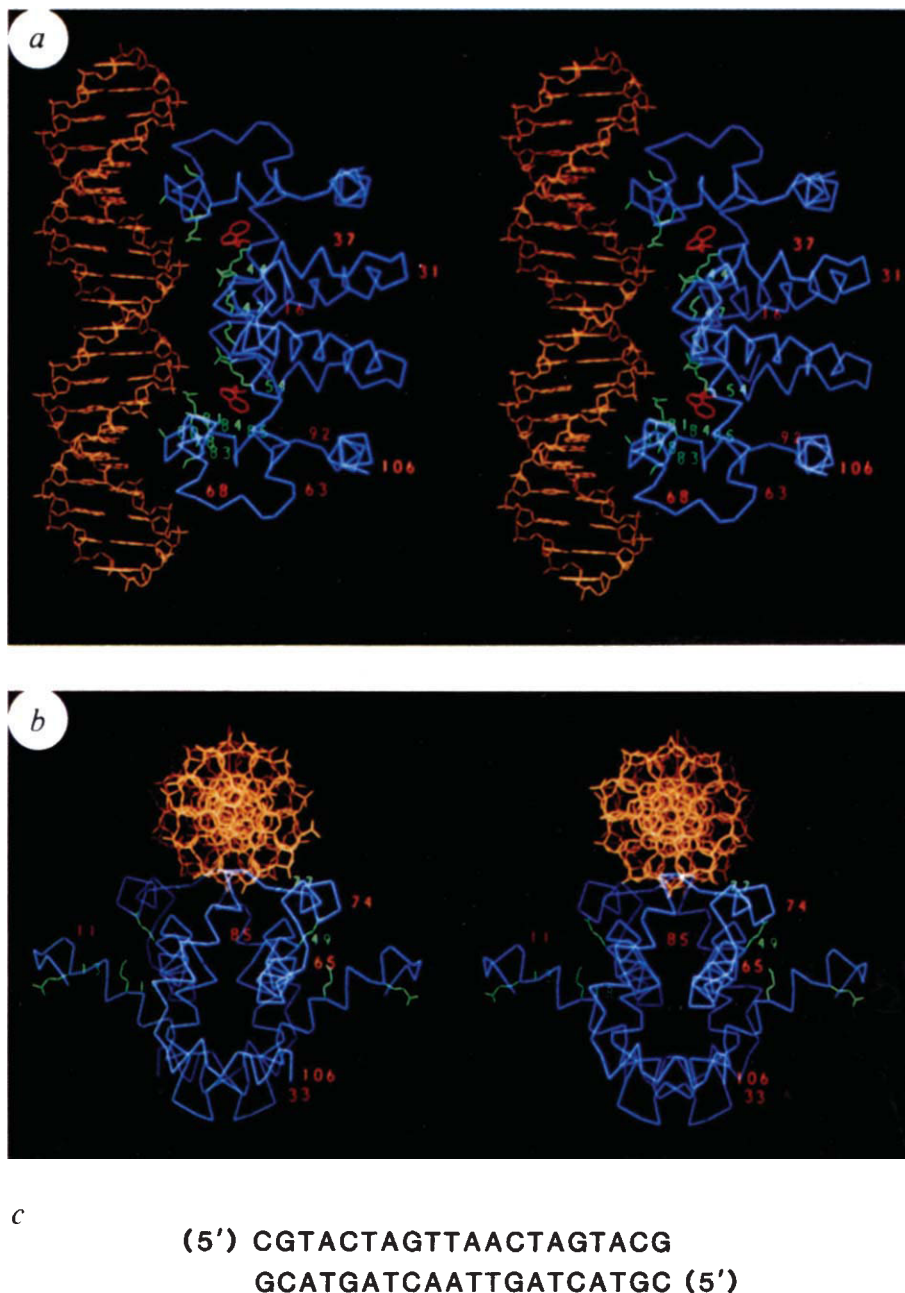
The interactive surface of the repressor is indicated by several observations. First, it contains as a prominent surface feature the helix–turn–helix DNA-binding motif that has both sequence and structural homology to the crystallographically determined DNA binding proteins *cro*⁶, λ repressor⁷, and CAP⁸. This element of the presumed interactive surface is separated from its dyad-related counterpart in the other subunit by one full turn of B-DNA. Second, this surface holds the pocket containing the L-tryptophan binding site. Third, this surface is lined with the amino acids that are altered in both the negative complementing and 'super repressor' missense mutants that presumably reflect decreased and enhanced operator binding, respectively (Fig. 4).

The complex was modelled by superimposing the 2-fold axes of repressor and operator and visually adjusting their separation and relative rotation about the common axis using computer graphics to give a good complementary fit between their matching surfaces. Figures 4 and 5 show the overall appearance of the modelled repressor/operator complex. In addition to the interaction of the helix–turn–helix elements with the major groove there is a more central contact surface in which the DNA backbones on each side of the minor groove approach the repressor surface close to the common dyad of the complex; however, the bases in the minor groove are not in contact with the repressor. In this modelled interaction the N-terminal end of the E helix is pointed into the major groove in primarily an 'end-on' manner so as to allow at least the first two residues, Ile 79 and Ala 80, to make close contact with the bases in the sequence-sensitive portion of the operator. A more confident description of this interaction awaits further refinement of the *trp* repressor structure and more precise modelling of the complex. However, it appears that the helix–turn–helix motif of the *trp* repressor is related to its operator more as in λ repressor²⁸ than as in *cro*²⁹. The much shorter D helix of *trp* repressor fits across the major groove between the ribose phosphate backbones.

Tryptophan shapes operator binding surface

The bound tryptophan appears to have at least two roles in activating the repressor, both of them primarily indirect. Being wedged against the side of helix E, the tryptophan ligand stabilizes the functionally critical orientation of the helix–turn–helix substructure that is responsible for operator-specific contacts.

Fig. 4 Modelled interaction between *trp* repressor and operator. **a**, A stereo pair of the complex, modelled as described in the text, viewed perpendicular to the helical axis of the idealized operator (yellow) and the dyad axis. The C α -backbone of the *trp* repressor dimer is shown in blue. The co-repressor L-tryptophan is shown in red. The amino-acid side chains of the wild type at the sites of negatively complementing missense mutants are superimposed and numbered in pale green; other positions are numbered in red. The C α positions are 2 mm to the left of the label and are all in the same subunit. **b**, Same complex as in **a** viewed along the helical axis of the operator. The amino-acid side chains of the wild type at the site of changes in super repressor mutants are superimposed and labelled in pale green; the L-tryptophan co-repressor is omitted for clarity. **c**, Consensus operator sequence used above.



Its second role is more local and results from the interactions of the bound tryptophan's carboxyl and amino groups with functional groups of the protein near the DNA-binding surface of the protein. The side chain of Arg 84 is clearly positioned by a hydrophobic contact of its aliphatic arm with the indole ring and a polar interaction of its guanidino group with the tryptophan's carboxylate. The guanidino group is thereby fixed in a specific way close to the sugar-phosphate backbone. At the same time the tryptophan's amino group interacts with the C-terminus of helix B (of the opposing subunit), presumably neutralizing the helix's intrinsic dipole³⁰. Were there no tryptophan bound, a likely place for the side chain of Arg 84 would be the site of the ligand's α -amino group. Although the heterocyclic N-1 of the indole ring may hydrogen bond to the DNA backbone through a water molecule, the charged functional groups of L-tryptophan do not appear to make direct contact with the operator. This assumes, of course, no major reorientation of the tryptophan ligand in the repressor/operator complex. The stereochemical details of tryptophan's activating role await the completion of current work on the crystal structure of the unliganded aporepressor and the inactive pseudorepressor

in which L-tryptophan is replaced by analogues such as indole propionic acid that contain no α -amino group⁵.

Amino acid changes due to *trpR* mutations

Kelly and Yanofsky¹⁰ have identified nine different nonsense mutations in *trpR*. Those occurring up to codon position 19 are simple recessives that presumably form short and completely nonfunctional peptides. A stop signal at position 68, however, produces the strongest of a class of negative complementing mutants, whose *trpR* translation product presumably forms inactive or weakly repressive hybrid dimers with the wild-type subunit. Figure 2 shows how the amino-terminal three helices are sufficient to stabilize a dimer when the opposing subunit is normal. Note that polypeptide segments in the environment of helices A, B and C are provided mostly by the opposing subunit. Thus, the tertiary structure of the first 62% of the polypeptide would appear capable of folding since almost all of its tertiary structural interactions are provided by the opposing subunit. This presumably allows a truncated polypeptide to form a stable, properly folded domain with an adequate intersubunit interface.

Figure 4a shows that the positions of changes in the polypep-

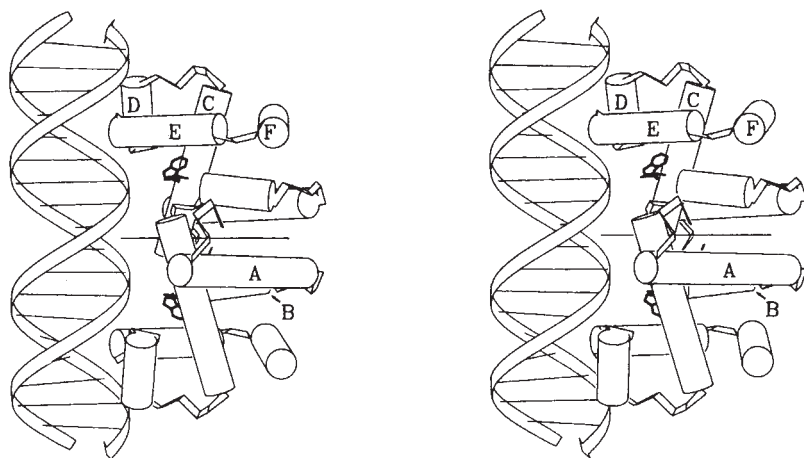


Fig. 5 Schematic representation of the *trp* repressor/operator interaction. The same complex as modelled in Fig. 4 except that the interaction is simplified by representing the repressor helices as cylinders and the DNA by a helical ladder in which the sides are the deoxyribose phosphate backbone and the rungs are the base pairs.

tide caused by negative complementing missense mutations are distributed on the surface of the repressor stretching from the helix-turn-helix region of one subunit to that of the other. Mutations occurring at codon positions 78, 80, 81 and 83 appear to disrupt critical recognition contacts with the operator's deep groove. Mutations at codon positions 44, 47, 54, 81, 84 and 85 change amino acids that surround the tryptophan binding site and may affect the mechanism by which the binding of L-tryptophan triggers repressor activity.

Kelly and Yanofsky¹⁰ also found 'super repressors' with mutational changes at codon positions 13, 18, 49 and 77 that exhibited substantial repressor response to the tryptophan even when the cells were almost starved for tryptophan. These altered amino-acid side chains face the operator as do the changes in the negative complementing missense mutants discussed above (Fig. 4b). In three of the four super repressor mutants Glu is converted to Lys. These changes are distributed in a surface region close to but not necessarily contacting the more central sequence-independent portion of the operator. In one mutant, Ala 77 is replaced by Val in the turn of the helix-turn-helix region. It is noteworthy that the more hydrophobic residue, Val, is markedly preferred at the equivalent position in other DNA binding proteins¹⁹. None of the altered amino acids in the super repressors are directly involved in L-tryptophan binding.

Flexible amino-terminal segment

Electron density for the first seven N-terminal residues is not as strong and contiguous as that representing the rest of the polypeptide backbone, suggesting that the N-terminal segment's position is not as well defined in the crystal structure as the rest

of the molecule. What can be seen of the N-terminal segments is folded back along the A helices (Figs 1, 5). If these N-terminal segments were freely mobile, model building shows that they could wrap around the DNA duplex and interact with the 'side' and possibly the 'back' of the operator. Evidence that this may occur comes from methylation protection experiments³¹ that show repressor protects the minor groove region on the back of the operator duplex. A similar situation has been observed for the amino terminus of the λ repressor where chemical protection studies suggest that a structurally ill-defined amino-terminal segment wraps around the operator²⁰.

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A survey for short-period pulsars

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All extensive pulsar surveys to date¹⁻⁵ have had sensitivities that deteriorate rapidly for periods P shorter than a few-tenths of a second, and none of them has had any appreciable sensitivity for $P < 30$ ms. For this reason, the unexpected discovery of two millisecond pulsars^{6,7} raised the question of the possible existence of a large population of very short-period pulsars, previously overlooked because of bias in the surveys. In an attempt to remove this bias, we have carried out an extensive new survey sensitive to pulsar periods as short as 4 ms. The search covered $\sim 3,725$ square degrees, mostly at galactic latitudes $|b| < 15^\circ$ and longitudes $15^\circ < l < 230^\circ$, with a sensitivity of 2–100 mJy depending on period, dispersion measure, and sky background temperature. We detected 87 pulsars, including 20 new ones, but found none with $P < 100$ ms. We conclude that the Galaxy does not contain a large population of pulsars with $4 < P < 100$ ms, unless their radio luminosities are substantially less than those of slower pulsars.

Our work, a continuation of the Princeton-NRAO pulsar survey⁵, was carried out with the 92-m telescope at Green Bank, West Virginia at a frequency of 390 MHz. At this frequency the telescope half-power beamwidth is 0.6° and the system noise temperature is 30 K, excluding sky background in the forward direction. We observed 14,195 distinct beam areas, of which 10,099 were at galactic latitudes $|b| < 15^\circ$, where most known pulsars lie. Each beam area was observed for 2.2 min. Signals from two orthogonally polarized feed antennas were amplified, converted to intermediate frequency, and passed through a filter bank spectrometer that provided 32 adjacent frequency chan-

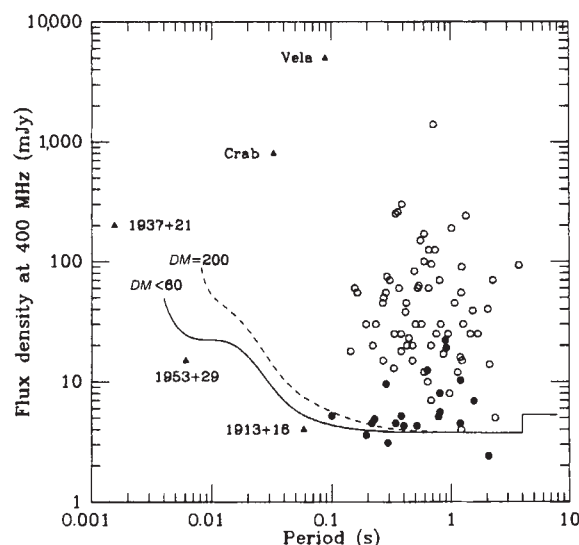


Fig. 1 Periods and flux densities of the 87 pulsars detected in this survey: ●, newly discovered pulsars; ○, previously known pulsars. The approximate sensitivity of the survey is shown for dispersion measures $< 60 \text{ cm}^{-3} \text{ pc}$ and $\sim 200 \text{ cm}^{-3} \text{ pc}$. For comparison, ▲ denote the periods and flux densities of the five fastest radio pulsars known.

nels, each of bandwidth 0.25 MHz. The orthogonally polarized signals at each frequency were detected, summed, smoothed with a 2.2-ms time constant, and sampled at 2 ms intervals. Recognizable interference and slow variations in signal level were removed by an online computer before the data were transferred to magnetic tape for later analysis. With a coarse quantization of three bits per sample, the survey data occupied more than 1,000 computer tapes.

The 2.2-min observation of a given beam area consisted of 65,536 samples from each of the 32 frequency channels. Data were processed using a two-dimensional Fourier transform technique^{8,9} to search for dispersed, periodic signals. With a dedicated computer equipped with a fast floating-point array processor, the required processing time was ~ 6 months. Twenty new pulsars were discovered and confirmed, and 67 previously known ones were detected one or more times. In Table 1 we present the coordinates, periods, dispersion measures DM , and approximate flux densities S of the new pulsars. Figure 1 illustrates the distribution of all 87 pulsars with respect to period and flux density. Complete results of the survey will be published elsewhere (G.H.S. *et al.*, in preparation).

We measured the sensitivity of the survey by injecting periodically-pulsed calibration noise into the receiver front end and subjecting the recorded data to the usual analysis procedure. Because the calibration signals were not dispersed, this test accurately simulated pulsars with $DM < 60 \text{ cm}^{-3} \text{ pc}$ (for which the smearing across a single 0.25-MHz channel is less than the post-detection time constant of 2.2 ms). We found the measured sensitivity and its dependence on period to be in satisfactory agreement with theoretical expectations^{5,9}. The calibration measurements are summarized by the solid curve in Fig. 1, scaled to a sky background temperature of 60 K. We also calculated the expected degradation of sensitivity as a function of dispersion measure; the result for $DM = 200 \text{ cm}^{-3} \text{ pc}$ is shown by the dashed curve in Fig. 1.

Our main purpose here is to document the fact, revealed in this survey, that pulsars with $P < 100$ ms are uncommon. For comparison purposes we plot in Fig. 1 the flux densities and periods of all five known pulsars with $P < 100$ ms. Our survey's sensitivity was sufficient to detect pulsars like the luminous Crab and Vela objects at distances as great as 8 kpc. (Neither of these pulsars was actually in the region searched, but the Crab pulsar

Table 1 Parameters of 20 newly discovered pulsars

PSR	$\alpha(1950)$	$\delta(1950)$	P (s)	DM ($\text{cm}^{-3} \text{ pc}$)	S (mJy)
0113+58	01 h 13 min	58° 49'	0.101437 ± 2	49 ± 8	5.2
0144+59	01 44	59 20	0.196321 ± 2	40 ± 8	3.6
0609+37	06 09	37 01	0.297982 ± 4	25 ± 7	3.1
1716-16	17 16	-16 28	1.5655 ± 4	43 ± 10	6.9
1726+00	17 26	00 02	0.38600 ± 3	39 ± 11	5.2
1740-14	17 40	14 00	0.4053 ± 2	128 ± 15	4.3
1759-04	17 59	-04 00	0.92148 ± 3	106 ± 20	19.1
1802+03	18 02	03 06	0.21871 ± 1	75 ± 11	4.5
1804-12	18 04	-12 00	0.52276 ± 2	120 ± 60	4.3
1811+02	18 11	02 29	0.79389 ± 2	104 ± 20	5.1
1847+13	18 47	13 28	0.3455 ± 4	57 ± 15	4.5
1849+12	18 49	12 57	1.20529 ± 2	70 ± 15	4.5
1858+01	18 58	01 57	0.288218 ± 2	93 ± 8	9.6
1902-00	19 02	-00 57	0.64318 ± 2	248 ± 20	12.4
1931+24	19 31	24 15	0.81368 ± 2	89 ± 20	8.0
2001+40	20 01	40 54	0.90506 ± 2	160 ± 30	22.1
2012+38	20 12	38 23	0.23019 ± 4	254 ± 40	4.9
2028+37	20 28	37 23	1.21681 ± 3	217 ± 35	10.3
2035+19	20 35	19 53	2.07437 ± 3	38 ± 25	2.4
2053+21	20 53	21 52	0.81518 ± 2	49 ± 20	5.6

Position uncertainties are 0.3° in each coordinate, period uncertainties refer to the last digit quoted, and flux densities are uncertain by $\sim 30\%$.

was detected easily when we observed it intentionally and carried out the normal search procedure.) Pulsars 1953+29 and 1913+16 are slightly below our detection threshold, but pulsars like them could have been detected at distances ≤ 2 and 4 kpc, respectively.

Figure 1 shows a conspicuous lack of detectable pulsars in the period range 4–100 ms. At most a few per cent of pulsars in the regions surveyed can have periods in this range and flux densities above our detection threshold. The number of short-period pulsars to be expected depends, of course, on one's preferred evolutionary model. The model of Gunn and Ostriker^{10,11} predicts few fast pulsars in any flux-limited sample, even if all pulsars are born with short periods, because the fast ones quickly spin down to $P \geq 0.3$ s while their luminosities remain essentially unchanged. Such a model would be in good agreement with our survey results, except that there exists increasingly persuasive evidence that the radio luminosities of pulsars are inversely correlated with period^{12–14}. Hence it is surprising that short-period pulsars, which tend to be the most luminous, have not been discovered in greater numbers—especially in the present relatively unbiased search. This result requires that many pulsars must be 'injected' into the population^{13,15,16} with $P \geq 0.5$ s. We are now inclined to take this view seriously.

Other evidence has been accumulating which suggests that fast pulsars of a different class from the Crab and Vela type are formed when neutron stars in binary systems are spun up by the accretion of mass and angular momentum from an evolving companion star^{16–22}. In particular, it seems likely that certain binary X-ray sources, especially those of the 'galactic bulge' type, may become millisecond radio pulsars when the accretion phase is over²³. Although we did not find any such pulsars, our survey does not contradict the idea because the volume of the Galaxy surveyed is still rather small and does not include the inner portions where most of the bulge-type X-ray sources lie. The expected period of a newly spun-up pulsar depends on its magnetic field¹⁹; neutron stars which still possess fields of $\geq 10^{12}$ G may become the 'injected' pulsars with $P \sim 0.5$ s, while those whose fields have already decayed to $\sim 10^8$ or 10^9 G may spin up to periods in the range 1–10 ms, where no survey has yet been done with appreciable sensitivity. It appears that full understanding of rapidly rotating pulsars will require surveys sensitive to periods as short as ~ 1 ms and dispersion measures up to ~ 300 cm⁻³ pc. We will discuss these conclusions more fully elsewhere.

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Liouville's theorem and the velocity field of galaxies

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Did the large-scale distribution of matter in the Universe evolve by a dissipationless process (such as hierarchical clustering) or is it the result of a strongly dissipative event (such as the 'pancake' scenario)? If the distribution of galaxies in the Universe can be treated as a dissipationless fluid, then an application of Liouville's theorem predicts a linear relationship between the observed galaxy density and the cube of the velocity dispersion as measured at any point in space. Evidence from existing cluster data is presented here which supports this hypothesis.

In any Friedmann–Robertson–Walker (FRW) cosmology, if the matter distribution (galaxies) is treated as a dissipationless fluid, then its phase-space is subject to the constraints imposed by Liouville's theorem; that is, the phase-space density must remain constant in time. An immediate result of this statement is that in the absence of any density structure, the velocity dispersion of the matter distribution must decay according to $\sigma(v) \propto R(t)^{-1}$, where $R(t)$ is the scale factor of the FRW cosmology, and $\sigma(v)$ is the velocity dispersion.

If structure is allowed to develop during the expansion, the velocity field of the matter must remain coupled to the local density. A trivial application of Liouville's theorem then predicts that:

$$\sigma(v) = \sigma_0 \left(1 + \frac{\delta\rho}{\rho} \right)^{1/3} \quad (1)$$

where $\sigma(v)$ is the velocity dispersion of the matter at the point where the density contrast, $\delta\rho/\rho \equiv \delta$, is measured, and σ_0 is the velocity dispersion where the contrast is zero (field dispersion). The highly textured density structure of the Universe offers an ideal place at which to make many local measurements. Particularly suitable for this task are rich clusters and small groups of galaxies.

Using the original counts and radii of Abell¹ to estimate density contrasts, and the compilation of Struble and Rood^{2,3} for velocity dispersions, 43 clusters can be found for which both density and velocity information exist. For low-density clusters the data on small groups of galaxies by Geller and Huchra⁴ were used to determine a median datum. The density of each cluster was estimated by assuming that the Abell counts are

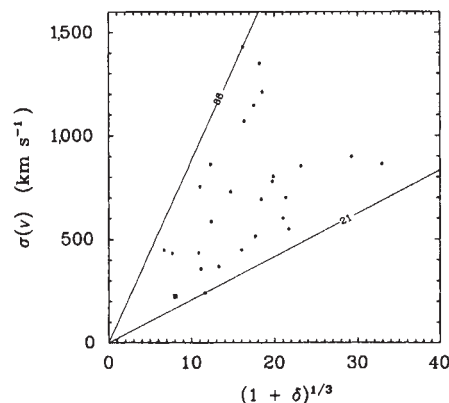


Fig. 1 Scatter plot of cluster velocity dispersion against the cube-root of the central density contrast for all clusters in Table 1 with radii < 3.0 Mpc. The solid-squares data point is the median for the small groups of Geller and Huchra.

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distributed within a sphere of the quoted radius. A density contrast was then found using the mean background galaxy density predicted by the general luminosity function of galaxies⁵⁻⁷. Explicitly, the density contrast is taken to be:

$$1 + \delta = \frac{\left(\frac{4}{3}\pi R^3\right) \left(\frac{N}{M_L}\right)}{\int_{-\infty}^{M_L} \phi(M) dM} \quad (2)$$

$$\text{with } M_L = (m_{10} + 1.5) - 5 \log(cz) + 5 \log(H_0) - 25 \quad (3)$$

where m_{10} is the magnitude of the 10th brightest galaxy in the cluster as given by Abell¹. The value $m_{10} + 1.5$ is an estimate, based on the adopted luminosity function, for $m_3 + 2$, which was Abell's criterion for his counts. The counts (N) and the radii (R) in which the counts were made, are from unpublished data taken from Abell's original compilation (R. Ciardullo, personal communication). The adopted luminosity function is

$$\phi(M) = 1.5(10)^{-3} X^{-0.25} e^{-X} \quad (4)$$

$$X = 10^{0.4(-20.9 - M)}$$

(A value of $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is used throughout this discussion, however, the density contrast is independent of this value.) Table 1 lists the cluster data used in the analysis. Figure 1 shows a plot of $\sigma(v)$ against $(1 + \delta)^{1/3}$ for all clusters in Table 1 with $R < 3.0 \text{ Mpc}$. Typical horizontal error bars may be as large as factors of 2. Errors in the velocity dispersion are considerably smaller. The restriction on the maximum radius was imposed to remove points with artificially low density contrast, which result when counts are divided into the maximum radius (averaging). For large clusters the data extend well beyond the cluster core where the velocity dispersion was determined, and where the contrast is constant. By imposing an upper limit to the radius of a usable cluster, a more accurate estimate of peak density contrast results. The solid-square data point represents the median for the Geller and Huchra groups. The median is plotted rather than the entire catalogue, as the catalogue suffers from severe background/foreground contamination and projection effects, whereas the median is a reliable representative measure for the groups.

The paucity of data points in Fig. 1 and the large scatter is disappointing, but the trend is clear. Rather than doing a linear regression on these data, an envelope has been drawn. The slopes of the two solid lines are 21 km s^{-1} and 88 km s^{-1} respectively. These data are consistent with a value of $\sigma_0 \approx 50 \text{ km s}^{-1}$. This value for the field galaxy dispersion, low by many estimates⁸⁻¹¹, is consistent with others^{12,13}. All of these measurements are, to some extent, contaminated by inclusion of data taken in regions where $\delta > 1$. It may indeed be the case that $\sigma_0 \approx 0$.

More important than the numerical value of the slope of equation (1) is its linearity. This linearity can be interpreted as evidence in support of a dissipationless scenario for the evolution of density structure, and the applicability of the Liouville theorem to the process. It is expected in this, as in any dissipationless process, that the Liouville theorem provides only an upper limit to the phase-space density. It may be argued that real clusters have experienced considerable diffusion in phase-space, resulting in velocity dispersions which are larger than the limiting value. If such is the case, the value of σ_0 derived from equation (1) is only an upper limit. This is an important point, as most estimates of σ_0 claim values considerably larger than the 50 km s^{-1} found here. Significant phase mixing may indeed be the case in the cores of some clusters, but many clusters appear to be only a few dynamical timescales in age. As the diffusion timescales are typically a few times longer than the dynamical times in the most violently relaxing systems (Lynden-Bell¹⁴), and much longer in more sedate systems, most

Table 1 Cluster data

Cluster	z	δ	$\sigma(v)$ (km s^{-1})	N	R (Mpc)	m_{10}
A98	0.1053	2,750	829	185	3.08	16.9
A154	0.0658	1,850	862	66	1.54	17.6
A168	0.0520	850	581	89	3.55	15.4
A194	0.0178	1,300	435	37	1.74	13.9
A262	0.0161	450	433	40	2.83	13.3
A272	0.0877	6,250	691	52	1.37	16.8
A347	0.0193	1,900	586	32	1.88	13.3
A397	0.0325	300	447	35	2.86	15.1
A399	0.0714	$3.5(10)^5$	864	57	1.12	15.6
A400	0.0231	1,550	242	58	2.26	13.9
A401	0.0746	6,050	1,349	90	2.48	15.6
A426	0.0183	450	1,308	88	6.08	12.5
A576	0.0392	6,350	1,211	61	1.91	14.4
A754	0.0519	550	1,191	92	4.56	15.2
A779	0.0226	1,350	755	32	1.99	13.8
A1060	0.0111	300	786	50	3.25	12.7
A1213	0.0474	$1.0(10)^4$	549	51	1.85	14.5
A1314	0.0340	9,750	701	44	1.66	13.9
A1367	0.0215	900	832	117	3.78	13.5
A1377	0.0509	1,400	358	59	1.79	16.6
A1452	0.0613	5,550	514	46	1.56	15.7
A1656	0.0232	50	880	106	10.28	13.5
A1775	0.0695	900	1,522	92	4.07	15.7
A1795	0.0620	7,700	778	115	1.70	16.0
A1904	0.0708	7,800	803	83	2.07	15.6
A2029	0.0767	4,250	1,430	82	2.25	16.0
A2052	0.0345	4,100	448	41	1.35	15.0
A2065	0.0722	4,400	1,070	109	2.82	15.6
A2142	0.0903	2,150	1,098	89	3.53	16.0
A2147	0.0356	5,400	1,148	52	2.43	13.8
A2151	0.0371	3,300	887	87	3.62	13.8
A2152	0.0374	1,950	1,244	60	3.87	13.8
A2197	0.0303	450	352	73	4.73	13.9
A2199	0.0303	550	808	88	4.73	13.9
A2247	0.0392	2,350	370	35	1.53	15.3
A2250	0.0654	3,200	729	52	1.53	16.5
A2255	0.0769	1,800	1,222	102	4.96	15.3
A2256	0.0601	350	1,257	88	5.87	15.3
A2319	0.0528	$1.2(10)^4$	854	68	1.34	15.4
A2589	0.0414	9,300	602	40	1.05	15.3
A2634	0.0312	$2.5(10)^4$	899	52	1.22	13.8
A2666	0.0265	350	261	34	3.62	13.8
A2670	0.0745	1,550	1,070	142	4.36	15.7

clusters are not expected to have experienced significant phase mixing.

The purpose of this letter is not to present incontrovertible proof of its naive hypothesis, but rather to stimulate interest in it. Many unpublished data on density and velocity dispersions of clusters are in the hands of researchers in the field. With a concerted effort, it should soon be possible to reconstruct Fig. 1 with considerably improved statistics. The validity of equation (1), if firmly established, would provide needed constraints on the evolutionary scenarios proposed for the growth of density structure in the Universe.

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Differential rotation and magnetic torques in the interior of the Sun

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The frequencies of solar oscillations can be measured with extreme precision and 5-min oscillations reveal the internal structure of the Sun¹⁻⁵. In particular, measurements of rotational splitting⁴ have provided the first reliable indications of the variation of angular velocity with radius⁶, while recent observations⁵ have yielded information on the variation with depth of latitudinal differential rotation. These results confirm theoretical predictions that the angular velocity decreases inwards in the convective zone^{7,8} but raise problems for dynamo models of the solar cycle. The suggestion that the core rotates with roughly twice the surface angular velocity has important implications both for the rotational history of the Sun and for other late-type stars, whose magnetic activity is closely correlated with rotation. Such a rapidly rotating core is hard to reconcile with the presence of any significant magnetic field pervading the entire radiative interior. We can only explain it by suggesting that the core contains a fossil field, unaffected by turbulence in the pre-main sequence Hayashi phase, that is decoupled from the rest of the star.

The solar angular velocity, Ω , is a function of radius r and latitude, but rotational splitting of the frequencies of sectoral harmonics is weighted towards the equatorial rotation rate⁴⁻⁶. At the surface ($r = R_0$), $\Omega = 2.90 \times 10^{-6} \text{ rad s}^{-1}$; Duvall *et al.*⁶ inverted the data showing that, immediately below the photosphere, Ω increases by 5–10%, before decreasing to a value slightly less than Ω_0 . For most of the convective zone ($0.9 > r/R_0 > 0.7$) there is no significant variation in Ω . In the outer part of the radiative zone ($0.7 > r/R_0 > 0.5$), Ω drops gradually to $\sim 0.9 \Omega_0$. The inner part shows more structure. There is an apparent local maximum of Ω around $r \approx 0.35 R_0$, followed by a dip and a steep rise to the core ($r < 0.2 R_0$). At $r = 0.15 R_0$ they find that $\Omega \approx 2\Omega_0$, and the angular velocity could continue to increase towards the centre of the Sun. Preliminary analysis⁵ of two-dimensional solar velocity images yields good qualitative agreement with these results, indicating also that the latitudinal differential rotation in the interval $0.7 > r/R_0 > 0.3$ is much smaller than that observed at the surface.

Numerical simulations of convection, in the Boussinesq⁷ and anelastic⁸ approximations, predict that Ω should be constant on cylindrical surfaces through much of the convective zone, so that $\partial\Omega/\partial r > 0$ at the equator—results broadly consistent with observations. In mean field dynamo models, dynamo waves propagate in a direction determined by the sign of $\alpha\partial\Omega/\partial r$, where α is proportional to the helicity. Within the convective zone, waves would propagate poleward, contrary to observation^{8,9}. This supports the idea that the field is generated in a shell at the interface between the radiative and convective zones¹⁰⁻¹³. At that level, α may change sign¹³ or $\partial\Omega/\partial r$ may be negative. In any case, the required variation in Ω is too small to be detected in the observations. Thus, although most dynamo models assume a radial variation of Ω at odds with the observations, there is no fundamental conflict.

On the other hand, the deduced behaviour of Ω in the radiative zone cannot be explained so readily. Consider first the existence of an isolated local maximum of Ω within the radiative zone. This seems incompatible with theory. The bump in Ω at $r \approx 0.35 R_0$ depends on the enhanced splitting for spherical harmonics of degree $l = 11$, which has been confirmed by two independent measurements^{4,5}. We have no adequate explanation for this splitting. It might be transient; alternatively, it could be a by-product of the daily observing windows (J. W. Harvey,

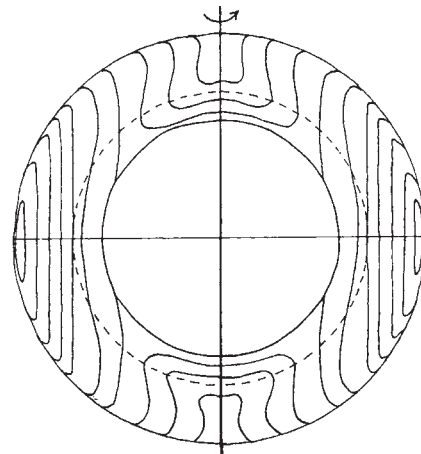


Fig. 1 Iso-rotational surfaces in the solar convection zone and in the outer part of the radiative zone. The dashed line indicates the base of the convective zone. The tendency to constant rotation on spheres for $r/R_0 < 0.7$ predicted by our model is in qualitative agreement with the results reported by Brown⁵.

personal communication) or a consequence of convection in giant cells, with horizontal dimensions comparable with the depth of the convective zone, but it is not clear how either mechanism could have such a marked effect.

Disregarding the bump, we may describe the radial variation of Ω in the radiative zone as solid body rotation (to within $\sim 10\%$) down to $r \approx 0.2 R_0$, with a steep increase to roughly $2\Omega_0$ at the centre. This sudden gradient is difficult to understand. The Sun has presumably been spun down by solar wind torques for at least its main sequence lifetime, and these torques act directly on the outer layers of the convection zone. Since the results of Duvall *et al.* show little if any differential rotation between the bottom of the convection zone and the outer radiative zone, there must be mechanisms that (1) enforce virtually rigid rotation from $r \approx 0.2 R_0$ to $r \approx 0.7 R_0$, and (2) couple the outer radiative and convective zones sufficiently well to mix angular momentum on the timescale for significant magnetic braking by the solar wind.

Consider the first of these two constraints. The timescale for Ekman spin-down seems too slow¹⁴. Could angular momentum be transferred by hydrodynamic instabilities? The ABCD instability, for which a marginally stable rotation curve has been derived¹⁷, has the lowest threshold of any of the diffusive hydrodynamic instabilities. However, the rotation curve deduced from the observations is extremely stable for $0.2 < r/R_0 < 0.7$, and hence such instabilities cannot be invoked in this region¹⁶. But magnetic fields are extremely efficient at transferring angular momentum¹⁸. In a perfectly conducting fluid, differential rotation generates an azimuthal field from a radial field B_0 and the Lorentz force exerts a torque that reduces the shear in angular velocity. The timescale τ for restoring rigid rotation is the Alfvén transit time $\tau_A = r/v_A$, where the Alfvén speed $v_A = B_0(4\pi\rho)^{-1/2}$ and ρ is the density. For a (modest) field of 1 G, $\tau_A \approx 10^4$ yr; to retain a rapidly rotating core for 3×10^9 yr, an implausibly small value of $B_0 \approx 3 \times 10^{-6}$ G (equal to the interstellar field) is required. The value of τ would be reduced if the azimuthal field were unstable on a timescale τ_1 that was significantly shorter than τ_A , but to have $\tau = 10^9$ yr with $B_0 = 0.1$ G would require $\tau_1 \approx 10$ yr, which seems extremely short. Theoretical arguments, therefore, imply that the radiative core should rotate rigidly both in radius and in latitude.

As for the second of these constraints, the presumed magnetic field in the radiative zone must be decoupled from the oscillatory field associated with the solar cycle, and so cannot transfer angular momentum between the radiative and convective zones. Instead, we suppose that the outer part of the radiative zone is hydrodynamically coupled to slowly rotating regions at high latitudes. The resulting equatorial acceleration diminishes with

decreasing radius until, by $r=0.5R_0$, the angular velocity is constant on spherical surfaces. Thus, contours of Ω should look as sketched in Fig. 1, which resembles the results obtained by Glatzmaier⁸ with different lower boundary conditions, and may be slightly modified in detail, but not in gross structure, by detailed inversions of the observations reported by Brown⁵. Such non-uniform rotation will drive a meridional circulation which may affect abundances of Li and Be.

The above considerations argue against the existence of a rapidly rotating core. In what follows we take as real the increase in Ω for $r < 0.2R_0$ derived from the splitting of the lowest degree modes, with $l=1$ (ref. 4), and try to explain it. The large-scale field that pervades the outer radiative zone cannot also thread the inner core; transport of angular momentum outwards would, as we have argued above, lead to virtual solid body rotation. This implies that the core (containing 30% by mass) was not mixed with the rest of the radiative zone at any time while the large-scale field was present in the outer radiative zone. As this magnetic field was either brought into the star during protostar formation, or generated by a dynamo once the protosun became convective and started to descend the Hayashi track, we conclude that the Sun was never fully convective.

In numerical studies of spherical collapse, the point where a protosun joins the Hayashi track depends on the duration of the accretion phase, which in turn depends on the initial conditions. If the initial state is centrally condensed, the protosun is fully convective¹⁹. Models starting with a homogeneous gas cloud, which collapses owing to the Jeans instability, lead to a convective zone that includes somewhat more than the outer 50% by mass^{20,21}. It has been argued that the position of the temperature maximum in the interior of a protosun does not remain at the centre, but migrates outwards owing to surface heating of the collapsed core by accreting matter from a disk²². As a result, deuterium starts to burn in a spherical shell enclosing $\sim 20\%$ of the mass; within this radius, no convection can occur. The evolution of a rotating magnetic protostar is bound to be even more complicated²³.

The existence of a rapidly rotating core, nevertheless, implies that in pre-main sequence evolution, convective mixing affects only the outer layers of what will eventually become the radiative zone of the main sequence star. The inner core, which should retain flux from the primeval field, is then magnetically decoupled from the rest of the star. As the star evolves, the core is constrained to rotate uniformly by this fossil field, but need not have the same angular velocity as the outer radiative zone. Measurements of the rotational splitting of solar p-modes may therefore reveal details of the pre-main sequence evolution of the Sun.

Thus, we can construct a plausible account of the rotational history of the Sun, which is summarized in Fig. 2. Unless there is a wide spread in initial angular momentum of solar-mass protostars^{24,25}, the variation of the surface rotation rate, $\Omega_s(t)$, at the equator with age can be derived from the measured projected rotation velocities of G stars in the Hyades^{26,27}, Pleiades²⁴ and α Persei²⁵, as well as of pre-main sequence stars²⁸: T Tauri stars (which lie on the Hayashi track) are relatively slow rotators²⁸, but contract as they approach the main sequence and, because the timescale for angular momentum loss due to stellar winds exceeds that for contraction, their angular velocities increase. Having descended to the main sequence, these stars apparently spin down rapidly during the next $\approx 3 \times 10^7$ yr and are then more gradually braked.

A shell enclosing 95% of the total mass corresponds to $r = 0.5R_0$ for the present Sun, so we expect the angular velocity to be independent of latitude and equal to $\sim 0.9\Omega_0$ (see Fig. 1). The rotation curve for this shell should lie just below that for the surface throughout the Sun's lifetime on the main sequence. When the Sun arrived on the Hayashi track, $\sim 10^6$ yr after the initial collapse, the outer convective zone contained 70% of the mass. Hence, we would expect the angular velocity on this shell to vary with latitude, with an equatorial value that is rather less than $0.9\Omega_s$.

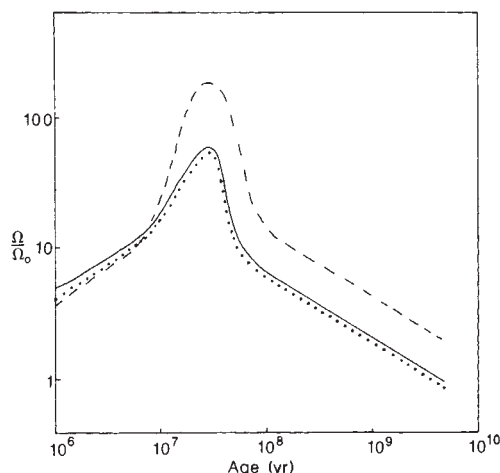


Fig. 2 The evolution of the rotation rate in the interior of the Sun, from the time when it evolved onto the Hayashi track until the present. The three curves show the equatorial angular velocity at the surface, Ω_s (solid line), on the spherical shell containing 95% of the mass (dotted line), and at the boundary of the inner core, containing 30% of the mass (dashed line). For the present Sun these correspond to $r/R_0 = 1.0, 0.5$, and 0.2 respectively. The maxima coincide with the arrival of the Sun on the zero age main sequence.

Finally, we come to the inner core containing 30% of the mass of the present Sun, which now rotates at twice the surface rate. Despite this relatively rapid rotation, the core cannot be fully isolated from the remainder of the Sun; if its angular velocity were to reflect that of the primordial Sun, the Sun would have been an abnormally slow rotator when at the age of similar-mass stars in, for example, the Hyades^{26,27} or Pleiades²⁴. Hence, the core must have lost angular momentum since the Sun arrived on the main sequence. Now the radial gradient of Ω deduced for the present Sun is sufficiently steep around $r \approx 0.2R_0$ for (slow) mixing by hydrodynamic instabilities to occur. At this radius there are significant μ -gradients, which have a strong stabilizing effect, especially for magnetic buoyancy instabilities. However, the ABCD instability¹⁷ is unaffected by such gradients (because the associated motions are largely horizontal) and so provides a mechanism for slowly transferring angular momentum while maintaining the magnetic isolation of the inner core. The latter occurs because, as the core magnetic fields are mixed in at the inner (core) boundary of the angular momentum mixing layer, the extreme efficiency of magnetic torques ensures that this inner boundary and the core come rapidly into co-rotation. Hence, the core de-spins not by a gradual reduction of the angular velocity gradient around $0.2R_0$, but rather by a gradual outward movement of the shear zone, coupled with a slow decrease in the jump in angular velocity as the core's angular momentum drops; thus magnetic fields need not mix throughout the shear zone. In that case, the core must have rotated at about twice the surface rate during most of the Sun's lifetime on the main sequence.

The variation of Ω with radius on the Hayashi track may depend on details of magnetic braking during the accretion phase²⁹. We suppose, however, that the inner core was linked to the convective envelope in much the same way as the outer radiative zone is now linked to the convective zone. Then the core would have rotated at a rate of $\sim 0.8\Omega_s$. As the Sun approached the main sequence, developing a strong central condensation, the core would have spun up relative to the exterior. On the zero age main sequence, therefore, the core must have rotated at more than twice the surface rate, before being rapidly decelerated by mixing instabilities at its outer boundary. This also implies that the magnetic fields linking the inner core to the convective envelope during most of the Hayashi contraction must have decoupled as the strong central condensation formed on the approach to the main sequence (possibly

through buoyancy instabilities of the strongly amplified fields in the shear layer separating the contracting core from the rest of the star). Thus we are led to the qualitative picture of spin down in the solar interior shown in Fig. 2. This differs significantly from the curves obtained by Endal and Sofia³⁰, who assumed that angular momentum was transferred by mixing instabilities in the solar interior, so that $\partial\Omega/\partial r$ was always negative. An alternative theory³¹ suggests that the rapidly rotating core developed while the Sun was on the main sequence, as a result of an oscillatory instability³² which redistributed the angular momentum.

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Boundary structures are formed by organic components of the Murchison carbonaceous chondrite

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The first cellular systems to appear on the Earth were presumably assembled from three molecular species: information-storing molecules capable of replication, enzyme-like catalysts structurally encoded by that information and able to enhance replication rates, and boundary-forming molecules which could encapsulate the system represented by the first two molecular species. Encapsulation would provide a microenvironment conducive to interaction of the molecular species involved, and would further serve to differentiate such systems according to their reproductive success. Boundary structures could also provide permeability properties useful for the directed transport of nutrient molecules required for growth and replication. The experiments reported here focused on non-polar molecules extracted from carbonaceous chondrites. Such molecules represent plausible models for the mixture of organic substances present on the early Earth. We found that certain components in the extract have physical properties which lead to the formation of boundary structures.

Considerable progress has been made toward establishing plausible models for replicating molecules and their interaction with catalytic molecules in the prebiotic environment¹⁻⁴. Less is known about mechanisms by which such systems could be encapsulated to form the first cells. Previous investigations⁵⁻⁷ demonstrated that phospholipids can be assembled under simulated prebiotic conditions, to form membranous vesicles. Even relatively simple lipids like fatty acids and other single-chain amphiphiles are able to assemble into membranes under certain conditions^{8,9}. Despite the success of these simulation experiments, it is not clear that molecules available on the early Earth would have properties required for self-assembly into encapsulating membranes.

Carbonaceous chondrites are useful in approaching such questions. These meteorites contain significant amounts of carbon, ranging around a few per cent by weight, and are apparently derived from larger planetesimals which underwent physical and chemical transforming processes ~4,500 million years ago. A major fraction of the carbon content is present as organic

compounds. These can be treated as plausible models of organic mixtures produced on the primitive Earth by geochemical processes or by late infall of meteoritic material. Aliphatic and aromatic hydrocarbons, monocarboxylic acids, amino acids and other biologically significant molecules have been convincingly demonstrated in such meteorites, and their isotope composition precludes a terrestrial origin^{10,11,12}.

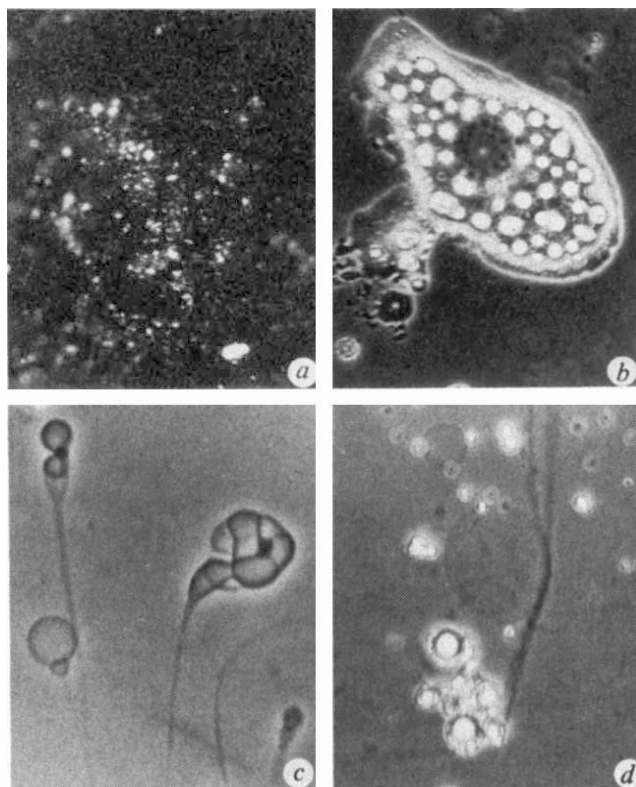
The present investigation assumed that organic compounds typical of those found in carbonaceous chondrites were in continuous interaction with early lakes and oceans. The experiments were directed toward determining whether any of the components were capable of self-assembly into boundary structures under such conditions. As boundary structures in aqueous environments are typically produced by non-polar compounds, carbonaceous chondrites were first extracted in solvent systems which would be expected to solubilize such compounds. Samples of the Murchison and Allende meteorites, both as powders and as solid material, were given by Dr Sherwood Chang, NASA Ames, Moffet Field, California. The Murchison chondrite is relatively rich in organics, while the Allende chondrite has little organic content and therefore served as a control for possible contamination introduced by the extraction procedure. The powders were extracted under conditions in which both polar and non-polar phases were present in order to permit partitioning of organic components between the two phases (see Fig. 1 legend).

Aliquots of a chloroform extract were analysed by infrared and ultraviolet spectrophotometry, acid-based titration, and light microscopic methods using a Zeiss epifluorescence microscope. A Wilhelmy surface balance (Cahn recording electrobalance and platinum plate) was used to obtain surface pressure isotherms.

The non-polar extracts had a distinct yellow colour when dissolved in chloroform, and were fluorescent, with excitation-emission peaks at 395 and 475 nm, respectively. It was important to show that this material was indigenous to the meteorite, and not introduced by the extraction process. The presence of fluorescent organic components in the interior of the Orgueil chondrite had been demonstrated previously by Alpern and Benkheiri¹³. To determine whether similar components were present in the Murchison material, surfaces of a 0.8-g solid sample were examined immediately after fracture, and after gentle smoothing by a diamond-coated polishing disk. Both fractured and smoothed surfaces showed numerous yellow fluorescent particles in the micrometre size range (Fig. 1a) as well as larger structures with deep red fluorescence. Only the yellow fluorescent particles were substantially reduced in num-

Fig. 1 Physical appearance of organic components of the Murchison carbonaceous meteorite. *a*, A solid sample (0.8 g) of the meteorite was first fractured, and the fracture face was smoothed by a diamond polishing disk. The facet was observed by epifluorescence microscopy. Yellow fluorescent particles ranging from the limit of resolution to $\sim 10\ \mu\text{m}$ were observed throughout the sample. *b*, Non-polar components were extracted from the meteorite with chloroform/methanol, and aliquots were dried on a microscope slide, then rehydrated with alkaline phosphate buffer, as described below. The non-polar components were present as viscous fluid droplets with numerous inclusions. The microstructure shown is typical of extracts from 11 separate samples. The droplets were highly fluorescent when observed with epifluorescence microscopy using blue excitation light. If the pH was reduced to ≤ 8 , the droplets solidified and decreased markedly in volume. *c*, *d*, Membrane structures could occasionally be observed as extrusions from the droplets shown in *b*, but only at alkaline pH ranges.

Methods. *b*, In typical experiments, 0.2–0.4 g of powder were stirred into 2.0 ml of aqueous phase, followed by 3.0 ml of chloroform/methanol (2:1). For extraction of solid samples, the samples were first crushed using a stainless-steel pestle in a heavy glass tube containing the aqueous phase. The mixture was then stirred briefly in a vortex mixer, followed by centrifugation at 500 g for 5 min to separate the two phases. The upper phase of aqueous methanol was removed by pipette, and the lower chloroform phase was decanted from the powder. In preliminary experiments, $\sim 0.4\ \text{mg}$ of non-polar material was extracted per g of meteoritic material at high pH ranges ($\geq \text{pH } 10$), while only one-tenth this amount was extracted at acidic or neutral pH ranges (pH 4–8). The experiments described here were carried out with extracts in which the aqueous phase was 0.1 M sodium phosphate, adjusted to pH 12 with 0.1 M sodium hydroxide. To prevent contamination, samples were handled with stainless-steel forceps, and all glassware was cleaned with chromic acid, washed with double-distilled and deionized water, then rinsed with redistilled chloroform/methanol (2:1).



ber following a brief chloroform rinse, and these presumably represent the *in situ* appearance of the organic components of the Murchison chondrite. Their size and distribution closely resembled those of the Orgueuil chondrite¹³.

Infrared spectra of dried samples prepared as KBr pellets were obtained with a Perkin-Elmer Fourier transform spectrometer. These showed absorption peaks at wavelengths characteristic of C—H stretching, C—O and carbonyl stretching, and O—H stretching and deformation. A broad absorption between wavenumber 2,000 and 4,000 was also present (Fig. 2). Titration of the non-polar components dispersed by sonication in water revealed the presence of substances with pK values of 6.5 and 9.3. These results, together with the infrared spectra, suggest the presence of carboxylate and phenolic groups. The C—H stretching peaks also indicate the presence of hydrocarbons, and the broad absorption is characteristic of aromatic polymers. These results are consistent with previously published organic analyses of the Murchison chondrite^{14,15} which reported the presence of aliphatic and aromatic hydrocarbons, monocarboxylic acids and phenolic compounds.

Aliquots of the chloroform extract were spread at air-water interfaces and surface-pressure isotherms were obtained. The non-polar components displayed surface activity, and readily spread to form a film which could be compressed to surface pressures as high as $28\ \text{dyn cm}^{-1}$ before collapsing. The area of the film ranged from $500\ \text{cm}^2$ per mg at high surface pressures to $4,500\ \text{cm}^2$ per mg at low surface pressures. (For comparison, a monolayer of a pure fatty acid such as palmitic acid ranges from $4,800$ to $6,000\ \text{cm}^2$ per mg over the same surface pressures¹⁶.) A possible explanation for the much larger range of surface area for the Murchison material is that the surface-active components spread from a second phase that is not surface-active and which exists as a bulk phase at the interface. The results described here are summarized in Table 1.

Many surface-active substances are also able to assemble into lamellar structures when they interact with aqueous phases, and often the lamellae produce stable membranes. Therefore, condi-

tions were established under which phase boundaries might be produced, and light microscopy was used to monitor the non-polar components as they interacted with various aqueous phases. In these experiments, $20\text{-}\mu\text{l}$ aliquots of the chloroform extract were dried on microscope slides, an equal volume of aqueous buffer was added and a coverslip applied. Under these conditions, the non-polar components formed yellow microstructures which adhered firmly to the glass. These microstructures were again highly fluorescent when viewed by fluorescence microscopy, and typically contained numerous inclusions which appeared to be separated from one another by relatively stable boundary layers that prevented fusion (Fig. 1*b*). Because earlier experiments had shown that there were titratable groups in the non-polar Murchison components, the properties of the microstructures were examined over a range of pH values. At alkaline pH ranges (≥ 9), the microstructures were viscous fluid droplets. If an acidic buffer was used, or if the pH was lowered to ≤ 8

Table 1 Properties of the non-polar organic components extracted from the Murchison carbonaceous chondrite (summary of observations from 11 extractions)

Non-polar organic content (11 extractions)	0.2–0.8 mg per g meteorite
Fluorescence spectrum (2 samples)	Excitation 395 nm, emission 475 nm
Infrared peaks and tentative assignments (2 samples)	Wavenumber 1,360 (O—H deformation) 1,425 (O—H deformation) 1,680 (C=O stretching) 2,965 (C—H stretching) 2,980 (C—H stretching) 3,250 (O—H stretching)
Surface area of film (3 samples)	$4,500\ \text{cm}^2$ per mg ($1\ \text{dyn cm}^{-1}$) $500\ \text{cm}^2$ per mg ($28\ \text{dyn cm}^{-1}$)
pK values (2 samples)	6.5, 9.3

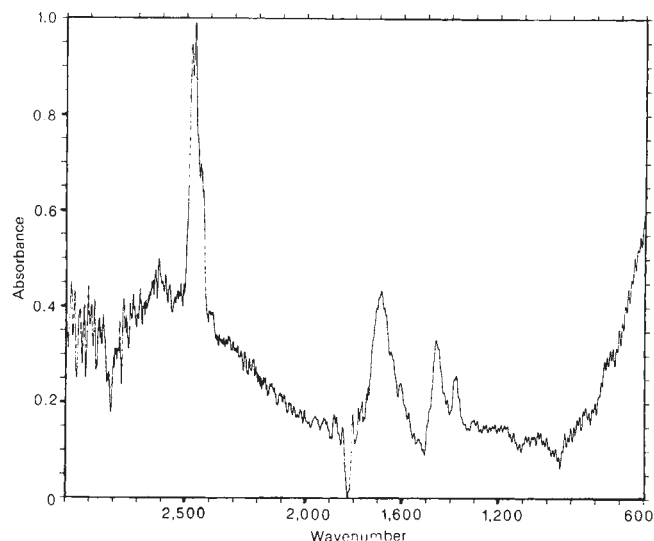


Fig. 2 Non-polar extracts of the Murchison meteorite were prepared as potassium bromide disks for infrared spectrophotometry. Spectra showed absorption bands at wavelengths characteristic of C—H, O—H and C=O stretching, and O—H deformation. The absorption between wavenumbers 2,000 and 4,000 is characteristic of fused aromatic ring systems.

by the addition of buffer at the edge of the coverslip, the droplets underwent a marked transition to solid phase, accompanied by a pronounced decrease in volume. Apparently the droplets were hydrated gels, and when ionic charges were reduced through titration, the gel lost its ability to retain water and shrank.

At high *pH* ranges (≥ 10) the droplets became increasingly fluid. In 10 mM NaOH, flow resembling convection currents occurred in the droplet interior, and in extracts from 3 of the 11 samples this was accompanied by formation of thin-walled structures at the interface. Over a period of several minutes, these structures grew to form large numbers of membranous vesicles and long strands which drifted away from the droplet with the flow of aqueous phase (Fig. 1c, d). The vesicles were clearly membranous, and had open interior volumes in which brownian motion of smaller particles could be observed. The largest vesicles were 50 μm in diameter.

To determine whether the vesicle boundaries were true barrier membranes, in some experiments the vesicles were formed in buffer solutions containing 1 mM 6-carboxyfluorescein as a fluorescent volume marker. The vesicles were then floated gently into a thin layer of Sephadex G-100 beads which were also enclosed under the coverslip. In these conditions, the dye was left behind by gel filtration, and vesicles containing the dye could be clearly observed deep in the gel layer. Apparently, the vesicles were formed from membranes sufficiently stable to contain carboxyfluorescein for at least several minutes.

Similar results were obtained with previously powdered Murchison material, and with solid samples that were extracted according to the protocol described earlier. When the Allende powder was extracted in parallel experiments, only a thin film was produced on the microscope slide. The film was not affected by alkaline solutions, and did not produce membranous material. The aqueous methanol phase, which also contained a yellow fluorescent substance, did not produce microstructures when dried and rehydrated with aqueous solutions.

Thus, certain components present in the Murchison chondrite have the ability to form two kinds of boundary structures at alkaline *pH* ranges. Microstructures in the form of viscous fluid droplets are produced at alkaline *pH* ranges; solid structures are produced at neutral and acidic *pH* ranges. In a sense, these droplets are analogous to the original 'coacervate' concept of Oparin¹⁷ in that they are complex mixtures of hydrated organic substances which form a gel when interacting with aqueous phases. The droplets contain a yellow pigment of unknown composition which is highly fluorescent. The photochemical properties of this pigment are well worth further investigation, as it is the first pigment molecule known to be derived from natural abiotic syntheses in the early solar system.

The second boundary structure takes the form of actual membranes, defined here as thin boundaries capable of acting as a barrier to free diffusion. These arise from the first structure

described above, and again require alkaline *pH* ranges for stability. Not all samples produced membranes under the conditions described here, perhaps because the membrane-forming compounds are not distributed uniformly throughout the Murchison material. Because of the small quantities available, and the complexity of the mixture, the composition of the membrane-forming components has not yet been determined. However, the fact that the membranes form and remain stable only at alkaline *pH* ranges suggests that they contain a titratable group, perhaps phenolic in character, which must become charged before it is able to enter into a membrane structure.

This represents the first report of boundary structures forming in aqueous phases from non-polar organic components of carbonaceous chondrites. Pflug¹⁸ has observed microstructures in the 0.1-micrometre range in Murchison samples prepared for electron microscopic examination, and Alpern and Benkheiri¹³ earlier demonstrated similar structures which they related to the organic content. Presumably, the non-polar material extracted here is derived from the structures observed *in situ*. Its relevance to the origin of membrane structures is not yet clear, however. There is no reason to expect that early membranes would have required alkaline *pH* ranges for stability. Furthermore, it is possible that some of the properties described here result from hydrolytic or oxidized derivatives of the actual Murchison components. The organic constituents of carbonaceous chondrites presumably formed under conditions of low hydration and essentially no free oxygen, and there may be unexpected reactivity when such components are exposed to an oxidizing, aqueous environment during extraction. Given these reservations, the formation of pigmented boundary structures from Murchison components is intriguing, and further analysis of their chemical composition may help to elucidate the early evolution of membrane structures.

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Seismic profiling the continental lithosphere

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Deep seismic-reflection profiling is successfully being used worldwide to study the structure of the continental crust¹⁻⁷. The MOIST⁸ profile recorded by BIRPS (British Institutions Reflection Profiling Syndicate) was the first to show that the structure of the upper mantle could also be imaged given a powerful source and long recording times. BIRPS has recently acquired an ultra-deep seismic reflection line, DRUM (Deep Reflections from the Upper Mantle). The DRUM line was designed specifically to investigate the reflectivity of the lower continental lithosphere and was therefore recorded to a two-way-time (TWT) of 30 s, giving a depth of penetration into the lithosphere of ~110 km. This profile has now revealed strong reflections which are unequivocally from the lower lithosphere, well below the base of the crust, and which are, as far as we are aware, the deepest and most continuous structures imaged in the upper mantle by multi-channel seismic-reflection profiling.

The profile is 184 km long and was recorded by GECO (Geophysical Company of Norway) in August 1984 and runs sub-parallel to MOIST off the north coast of Scotland (Fig. 1). The lithospheric thickness of offshore Scotland is not well determined but probably lies between the 80-90-km thickness of the North Sea⁹ and the 110-190-km thickness of onshore Britain¹⁰. The recording time (and hence depth) of the survey was limited to 30 s by the need to maintain a speed through the water of around 5 knots (2.5 m s⁻¹) and to shoot every 100 m. At slower speeds the hydrophone streamer becomes unstable, and a greater shot interval would lower the already low fold of cover to an unacceptable value. The seismic source used consisted of an areally extensive tuned airgun array containing 46 airguns with a total volume of 140 litres. We believe that the large size and high quality of this array were the most important features of the experiment, allowing us to collect good data at very long travel times. Reflections were recorded by a 3-km streamer with 60 channels towed at 17 m depth. The shot spacing used, 100 m, provided 15-fold data with a 25 m Common Depth Point interval.

Figure 2 shows an unmigrated line drawing of the DRUM profile prepared from a preliminary stack of the data. The crustal structure is broadly similar to that revealed by the nearby MOIST line^{8,11} and will be reported elsewhere. Figure 3 demonstrates qualitatively the effect of depth migrating the major sub-crustal reflectors and includes both a simplified stack and a depth section. Migration was performed by ray tracing through a simplified velocity model of the lithosphere; curvature of the rays by refraction was included¹². Migration of the time section

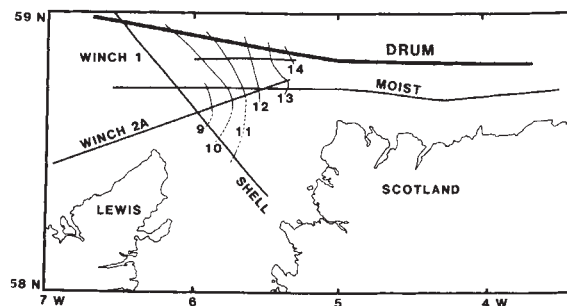


Fig. 1 Location of DRUM 30-s profile. The locations of other BIRPS deep seismic profiles, MOIST, WINCH 1 and WINCH 2A are shown as well as a 15-s profile collected by Shell Expro UK Ltd. The depth in seconds TWT to the Flannan Thrust is contoured to 14 s to show the three-dimensional structure.

into depth is difficult for deep reflection data but is essential for the proper spatial location and interpretation of reflectors¹³.

Four major sub-crustal groups of reflectors can be seen: the Moho at the base of the crust around 8-9 s; a sub-horizontal reflector superficially similar to the Moho at around 13-15 s; an easterly dipping sequence (the Flannan 'Thrust')⁸ running from the Moho down to at least 27 s and possibly 30 s; and a 15-km-long banded zone at 23 s. A section of each reflector is shown in Fig. 4. We believe all these events represent primary reflections from deep structures which are not significantly out of the plane of the section. We have examined multiple reflections, side-swipe, S-wave conversion, reflected refraction and diffraction effects as possible explanations for these events. The shape and amplitude of the reflections, their move-out on both common-shot and CDP gathers and the three-dimensional control provided by the MOIST and WINCH lines allow us to rule out most of these explanations. It remains a possibility, however, that the sub-horizontal events at the eastern end of the profile could be caused by side-swipe reflections. We intend to check this by further data collection in the area.

Along most of the DRUM profile the Moho can be seen as a bright reflector at the base of a seismically layered lower crust (Fig. 4b). Wide-angle observations suggest that the Moho is particularly sharp in this area¹⁴. At the western edge of the section, the Moho is less distinct and is interpreted to correspond to the base of the reflections in the lower crust¹⁵. The TWT to the Moho varies between 8 and 9 s (24 and 27 km) and is deepest in the east. Several refraction and wide-angle reflection experiments have been performed in this area¹⁶⁻¹⁸ and show average crustal velocities of around 6-6.4 km s⁻¹ and a sub-Moho velocity around 8 km s⁻¹. When the results of these are converted to normal incidence two-way travel time, they all predict Moho reflections at 8-9 s. Taken together they also indicate that the Moho deepens to the east.

The most exciting structure revealed by the DRUM line is the Flannan Thrust. We have now observed this event below

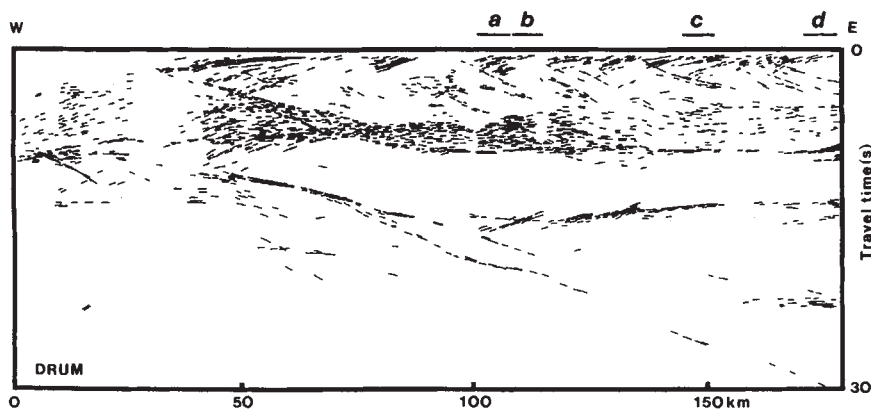


Fig. 2 Line drawing of DRUM profile based on a preliminary stack of the data. The section is in TWT and is true-scale at a velocity of 5 km s⁻¹. The rotated half-grabens of the upper crust and the reflective lower crust and Moho are shown as well as the Outer Isles Fault (the Outer Isles Thrust of previous BIRPS papers). Within the mantle are seen three major features: the east-dipping Flannan Thrust; a sub-horizontal reflector at 13-15 s between 80 and 180 km and the 15-km-wide zone of horizontal reflections at 23 s at the eastern end of the section. The locations of selected sections, a-d, shown in Fig. 4, are indicated at the top of the profile.

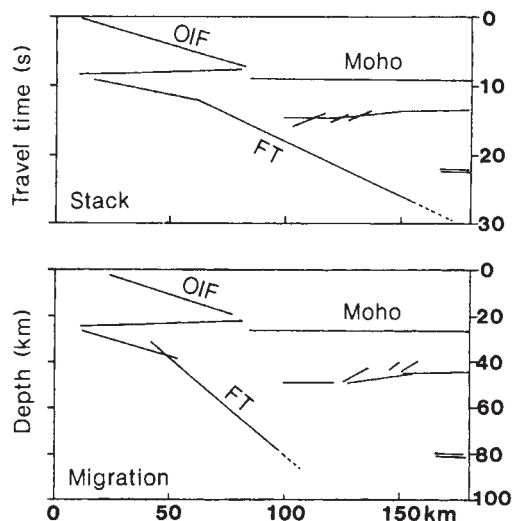


Fig. 3 A stack section in time and a depth profile produced by depth migration, showing qualitatively the effect of depth migration on deep reflectors. The major reflections are shown schematically as linear segments. All segments are repositioned in the depth profile constructed using a simple two-dimensional velocity model. The dipping reflectors become significantly steeper and migrate up dip. OIF, Outer Isles Fault; FT, Flannan Thrust.

the Moho on five deep profiles in the area (Fig. 1)^{8,11,19,20}. These profiles together show an easterly-dipping structure which originates in the lower crust, cuts the Moho, and may run off the bottom of the 30 s DRUM line. After migration, the structure has an average dip of 35° and extends to a depth of at least 75 km and possibly to 85 km (Fig. 3). The event is strongly reflective (Fig. 4a) and is non-planar, with a variable local strike and dip; on the DRUM line it appears to split into two parallel events (Fig. 2). Contours in time of the structure (Fig. 1) also show a non-planar three-dimensional structure. South of WINCH 1, the structure strikes roughly north-south (N-S) and is below and sub-parallel to the upper crustal Outer Isles Fault. To the north, the structure diverges from the N-S crustal structural trends to strike nearly NW-SE. The Flannan Thrust flattens to nearly horizontal in the lower crust and is nowhere seen above 7 s. We do not believe it cuts the upper crust or reaches

the surface. Indeed, although we often observe strong dipping reflectors on BIRPS profiles, both within the crust and upper mantle, we have never seen a reflector which runs unambiguously from the near surface to below the Moho.

Since the Flannan Thrust does not outcrop and is considerably below the reach of even the deepest borehole we must use indirect evidence to identify it and at present our conclusions are speculative. The geometry of the feature, particularly the dip, the general reflective character, the fact that it cuts and may offset the Moho, and the existence of similar features on parts of the WINCH¹⁹ and SWAT²¹ data and in eastern Australia²², suggest that the reflections are probably from a ductile fault or shear zone within the lower lithosphere. If this feature is indeed a zone of localized strain, we would expect it to have developed as a structure during either the Caledonian orogeny or later Mesozoic extension. If it formed as a thrust fault, it may have been reactivated during extension. Unfortunately, nowhere is an unambiguous offset of the Moho seen. A possible alternative explanation is that the reflections are coming from a remanent pre-Caledonian subduction zone. There is, so far as we are aware, no geological evidence for the existence of such a feature. However, a subduction zone is the one structure which all earth scientists would recognize as a roughly planar dipping structure that cuts the lower lithosphere.

We had expected to see the Flannan Thrust on the DRUM line, although perhaps not to such spectacular depths. In contrast, the sub-horizontal event seen below the Moho at about 13–15 s (45–50 km depth) was completely unexpected. This event is very strong and relatively continuous for ~100 km. Although it is difficult to determine the absolute strength of deep reflections, it is clear that this deep event is at least as strong and continuous as the Moho in this area. Figure 4c shows a stack over part of this event; the Moho in Fig. 4b is shown for comparison. At present, we have no cross-line control on the event and its cross-dip is undetermined, although the lack of any observable move-out is sufficient to rule out the possibility that the event is a gross side-swipe from a surface structure.

In detail, a structure is revealed which dips gently (2°–5°) to the west and is cross-cut by more steeply dipping (30°) features. After migration (Fig. 3) these cross-cutting events migrate above the flatter structure and appear to sole into it. It is also possible that the upper limb of the Flannan Thrust soles into this same structure from the west. Surprisingly, we did not observe analogous reflections on the MOIST line which is only 15 km to the south. This would appear to imply a cross dip of at least 20°, or that the event becomes less reflective to the south.

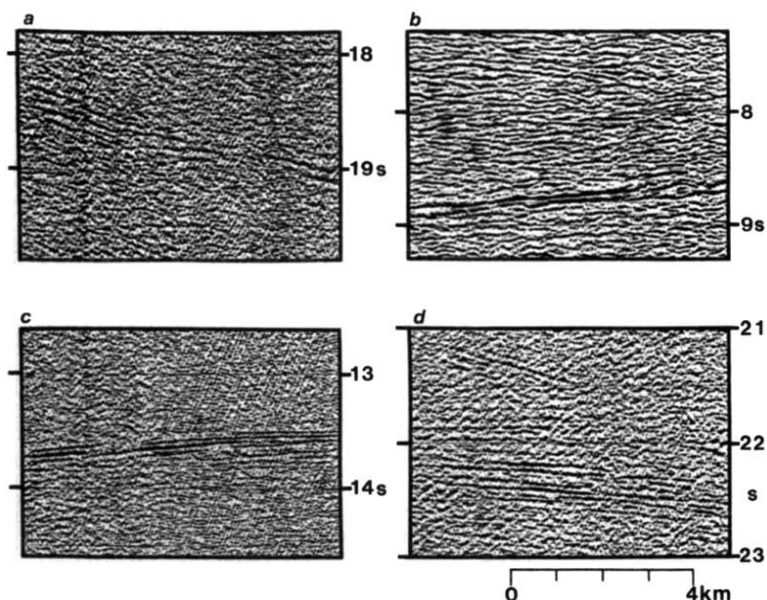


Fig. 4 Sections of the unmigrated preliminary stack showing: a, the Flannan Thrust; b, the Moho and reflective lower crust; c, the mantle reflector at 13–15 s; and d, the zone of reflections at 23 s.

However, the source used on the MOIST line was only about one-eighth as powerful as that used on DRUM and this may explain the absence of the event on MOIST.

Interpretation of this structure is even more speculative than for the Flannan Thrust. Deep mantle reflections may be produced in several ways, for example from a lithological boundary, a phase change, a zone of localized strain, a change in anisotropy or even a rheological boundary. We believe the most likely explanation is that the reflections are from a sub-horizontal shear zone, that is, a detachment or decollement surface within the upper mantle. Such a shear zone will probably be structurally related to the various dipping features seen in the area including the upper limb of the Flannan Thrust. The opposing dips of the two structures, however, will cause an interesting geometrical problem for any structural interpretation. Wide-angle observations to the south of the DRUM line indicate the existence of a reflector at a depth of around 55 km (ref. 23). The wide-angle results suggest that this may correspond to a change in velocity from 8.0 to 8.2 km s⁻¹. However, both the depth and velocity are poorly constrained.

The deepest (unambiguous) reflections seen after migration on the DRUM line are a short layered sequence seen at 23 s (Fig. 4d) at the extreme eastern end. The deepest of these is at around 85 km depth (Fig. 3). Again, these horizontal reflections may be due to some kind of compositional layering or phase change within the lowermost lithosphere. These reflections are similar in appearance to the highly reflective layered lower crust^{21,24} seen on much of the BIRPS data. It has been suggested²⁵ that the layering in the lower crust is due to a ductile fabric produced by pervasive shearing and that the top and bottom of the layering mark a (palaeo-)rheological boundary. Part of the motivation for the DRUM line was to look for similar layering in the lower lithosphere which may also be subject to pervasive shearing. The localized layering seen at the eastern end of DRUM is the only example of this seen on the line and we do not know what it represents. The presence of the Flannan Thrust to depths of 75 km or more suggests that the upper mantle is not pervasively sheared above such depths.

The observations discussed here are unique, and we have presented them primarily as a basis for informed speculation by other authors. The data demonstrate that the lower continental lithosphere can be structurally complex and that it may contain good reflectors which are continuous for at least 100 km. The results seem to show that the upper mantle can support relatively localized strain even near the base of the lithosphere. We hope to shoot even deeper data in this area to determine the ultimate fate of the Flannan Thrust as it approaches the asthenosphere and to try to observe reflections from structures associated with the lithosphere–asthenosphere boundary.

BIRPS is funded by the Natural Environment Research Council under the Deep Geology Programme. The data were acquired and are being processed for us by GECO. We were impressed by the quality of their acquisition system and ability to undertake an unusual and difficult project. We believe the high quality of these data reflects their efforts. Shell Expro UK Ltd provided access to their deep lines in the area and we thank them for their continued assistance to BIRPS. Copies of the DRUM profile may be purchased for the cost of reproduction from British Geological Survey, Edinburgh.

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Was the Greenland ice sheet thinner in the late Wisconsinan than now?

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Ice of Wisconsinan origin which constitutes the basal layers of the ice caps in arctic Canada and Greenland flows three to four times more readily than the Holocene ice above. A model based on simple ice sheet profile theory is set up for the thickness response of the interior ice sheet regions to the progressive thinning of this soft layer. The model is applied to calculate the thickness response of the Greenland ice sheet at the locations Dye 3 and Crête, and of the Devon Island ice cap in arctic Canada. It is concluded that the mechanism contributes significantly to the thickness change of the Greenland ice sheet, presently at a rate of about 1 cm yr⁻¹ and that this rate of change will persist potentially over thousands of years to come. As regards the Devon Island ice cap, most of the estimated 15% thickness increase has already been accomplished. A further consequence is that in the late Wisconsinan, ice thicknesses of the interior regions of the Greenland ice sheet were likely to have been no greater and possibly even less than at present, in spite of the larger geographical extent of the ice sheet. It is argued that the glacial-interglacial cycles of accumulation rate and ice temperature are likely to enhance this ice thickness variation.

Deformation measurements on bore holes drilled to bedrock through ice caps in arctic Canada and through the Greenland ice sheet have documented that the deep ice layers deposited in Wisconsinan time deform three to four times more readily than the above lying ice of Holocene origin^{1–4}. Deformation tests on specimens sampled from ice cores generally confirm this conclusion⁵. The Wisconsinan ice is characterized by a marked decrease in ice crystal size^{6–8}, and by up to 50 times more microparticles than in Holocene ice^{6,9–11}. These characteristics have been suggested as the cause of the relative softness of Wisconsinan ice^{2,3}, although a change in ice fabrics towards a vertical, single-pole crystal-axis orientation has also been considered a possible explanation⁵. However, even though a change in ice fabrics at the Wisconsinan-Holocene transition has been reported for some ice cores^{1,7,8}, the change is not as marked as those of crystal size and impurity content, and is even absent in some cores^{2,6}.

Whatever the explanation, the different flow properties of Wisconsinan and Holocene ice have consequences for the long-term ice thickness change of the ice caps. This is because a given ice flux can pass a given location with a smaller ice thickness, the softer the ice. Any internal horizon in the central regions of an ice sheet is continuously displaced downwards relative to the surface, due to flow induced thinning of the ice layers, compensated by supply of ice from snow accumulation at the surface. Therefore, the transition between hard Holocene ice, the deposition of which started at the surface some 10,000 years ago, and soft Wisconsinan ice has ever since migrated downwards with the consequence that an increasing portion of the ice sheet has come to consist of hard ice. This implies a potential

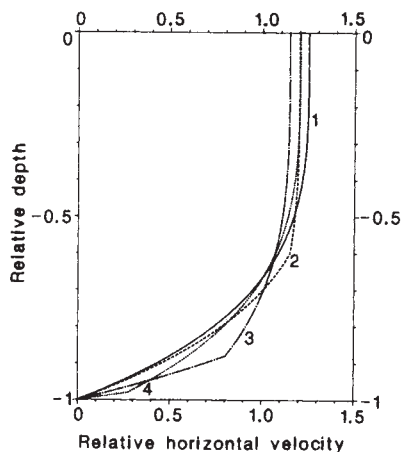


Fig. 1 Horizontal velocity profiles for different values of the relative depth z_T/H to the soft/hard ice transition. (1) $z_T/H = 0$ or $z_T/H = -1$ (representing an ice sheet consisting of either soft or hard ice throughout). (2) $z_T/H = -0.61$ (representing present-day Crête conditions). (3) $z_T/H = -0.88$ (representing present-day Dye 3 conditions). (4) $z_T/H = -0.97$ (representing present-day Devon Island ice cap conditions).

gradual increase of ice thickness in the central regions of the ice sheet. The actual response, however, will depend also on other factors such as changing ice flux caused by changing mass balance¹³ or changes of ice temperatures, induced by climate variations at the ice sheet surface¹⁴.

In this paper the ice thickness response to the gradual thinning of the soft basal ice layer and the accompanying thickening of the upper hard ice layer is considered in isolation. A simple model is applied, which is nevertheless believed to account for the more important aspects of the problem.

We shall start by calculating the ice flux in terms of ice thickness (H), surface slope (ds/dx), ice flow law parameters (n) and (A), depth to transition between hard and soft ice ($-z_T$), and enhancement factor (E). The enhancement factor is the degree by which the deformation rate of soft Wisconsinan ice is increased as compared to hard Holocene ice when subjected to the same stress and temperature. Assuming plane flow and neglecting the influence of the normal stress deviators on the ice flow, the vertical shear rate in the ice sheet may be expressed as¹²

$$\partial u / \partial z = 2A\tau_{xz}^n \quad (1)$$

where u is horizontal velocity, z vertical coordinate, τ_{xz} shear stress, and A and n ice flow law parameters¹².

If the ice thickness is assumed to vary slowly with the horizontal coordinate x , then τ_{xz} is to a good approximation distributed linearly with depth ($-z$) according to the equation

$$\tau_{xz} = \rho g z \, ds/dx \quad (2)$$

z is zero at the surface and is positive upwards; s denotes surface elevation, ds/dx surface slope, ρ ice density, and g gravitational acceleration.

The flow law parameter A in equation (1) depends on ice temperature, density, impurity content, crystal size and fabric¹².

Table 1 Ice thickness and accumulation rate at study sites

Site	Position		Thickness	Accumulation rate
	Lat. (N)	Long. (W)	(m)	(m of ice per yr)
Dye 3	65° 11'	43° 50'	2,000	0.55
Crête	71° 07'	37° 19'	3,000	0.28
Devon Island	75° 20'	82° 30'	300	0.23

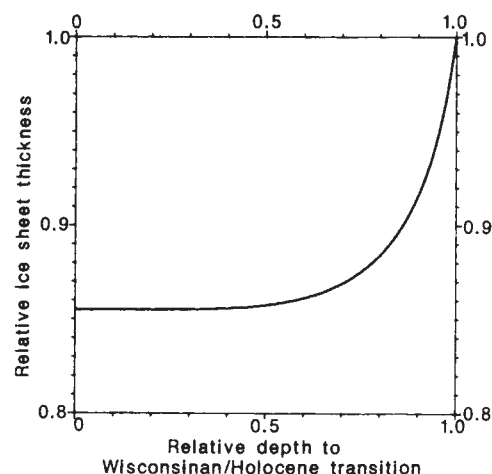


Fig. 2 Ice-sheet thickness relative to the thickness when the ice sheet consists of hard ice throughout as a function of relative depth to soft/hard ice transition.

These quantities may all vary with depth. However, in this study the only variation to be considered is a step-change in A at a depth ($-z_T$) corresponding to the Holocene-Wisconsinan transition, that is, $A = A_0$ for $z_T < z \leq 0$ and $A = EA_0$ for $-H \leq z < z_T$. This means that the influence on the velocity distribution of e.g. the temperature-depth profile is neglected.

With this assumption the horizontal velocity profile may be obtained from equations (1) and (2) by integration. Assuming the ice sheet to be cold-based (no basal sliding), we get:

$$u(z) = 2A_0(-\rho g \, ds/dx)^n H^{n+1} \phi(z/H) \quad (3)$$

where

$$\phi(z/H) = \begin{cases} E/(n+1)[(-1)^{n+1} - (z/H)^{n+1}] & -H \leq z \leq z_T \\ 1/(n+1)[E(-1)^{n+1} - (E-1)(z_T/H)^{n+1} - (z/H)^{n+1}] & z_T \leq z \leq 0 \end{cases}$$

In Fig. 1 velocity profile functions $\phi(z/H)$ are plotted for different values of z_T .

Integration of equation (3) with respect to z from $-H$ to 0 yields the ice flux:

$$Q = 2A_0/(n+2)(-\rho g \, ds/dx)^n H^{n+2} \times [E - (-z_T/H)^{n+2}(E-1)] \quad (4)$$

As explained above, z_T decreases steadily with time. Consequently, since we assume that the mass balance of the ice sheet and therefore also the ice flux Q be constant, the term $(-\rho g \, ds/dx)^n H^{n+2}$ must change with time so as to compensate for the change in z_T . If we disregard changes in the horizontal extent of the ice sheet and furthermore introduce the simplification that the ice sheet base is horizontal, then the surface slope ds/dx is to a good approximation proportional to the slowly changing ice thickness H . Therefore, from equation (4) we find that

$$H_{z_T}/H_{-H} = (E - (-z_T/H)^{n+2}(E-1))^{-1/2(n+1)} \quad (5)$$

where H_{-H} is the ice thickness when $z_T = -H$ (when the ice sheet is composed throughout of hard ice). This relationship is plotted in Fig. 2.

As can be seen from Fig. 2, an ice sheet composed of Wisconsinan ice is about 15% thinner than an ice sheet consisting of Holocene ice, everything else being equal. The thickness change is a non-linear function of the relative depth to the transition between soft and hard ice; when the transition is halfway down, the thickness change is hardly detectable and even when the soft ice layer constitutes only 10% of the ice thickness, only about half the thickness change has been accomplished.

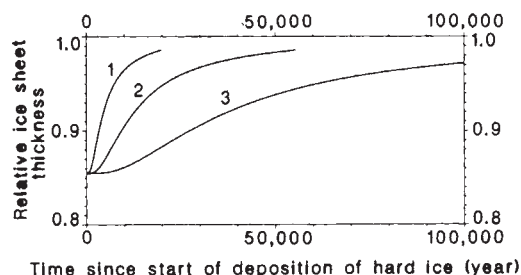


Fig. 3 Response of relative ice sheet thickness to gradual thinning of soft basal ice layer. The thickness is relative to the value that corresponds to an ice sheet consisting of hard ice throughout. (1) Represents Devon Island ice cap ($H = 300$ m, $a = 0.23$ m yr⁻¹), (2) Represents Dye 3 (Greenland ice sheet) ($H = 2,000$ m, $a = 0.55$ m yr⁻¹). (3) Represents Crête (Greenland ice sheet) ($H = 3,000$ m, $a = 0.28$ m yr⁻¹).

This is a demonstration of the relative importance of the deep layers for ice sheet dynamics in general.

It is interesting also to follow the thickness change as a function of time. This can be done rather simply for the central regions of an ice sheet, where the vertical velocity distribution $v(z)$ and the transition depth z_T are to a good approximation independent of x . There, $v(z)$ can be calculated approximately for any value of the transition depth z_T by combining equation (3) and the equation of continuity. Next z_T may be found as a function of time by integrating the equation

$$\frac{dz_T}{dt} = v(z_T)$$

Besides being dependent on n and E , the solution also depends on the ice thickness H and the accumulation rate a . For $n = 3$ and $E = 3.5$, Fig. 3 shows the ice thickness response for three different choices of H and a , corresponding to the conditions at Dye 3 and Crête on the Greenland ice sheet, and to those prevailing on the Devon Island ice cap in northern Canada.

The response curves in Figure 3 are very different: at present, that is 10,000 years since the start of the deposition of hard ice, the Devon Island ice cap has accomplished most of its thickness change (about 35 m of a total of 45 m), Dye 3 about one third only (100 m of a total of 300 m), and at Crête the change in thickness has hardly commenced (20 m of a total of 450 m). This large difference in response is due to the large differences in the depth to which the hard-soft ice transition has migrated into the ice sheet at the locations considered.

The modelling predicts the 10,000-year age-horizon to be found at the positions shown in Table 2. As regards the Dye 3 and Devon Island locations the model results can be compared to observed depths of the Wisconsin/Holocene transition, found by various ice core investigations to be 250 m and 5 m above the base, respectively^{3,6}.

Considering the approximate character of the modelling, the above agreement is satisfactory and supports the calculated present rate of change of ice thickness for the three locations of 1.2, 0.5 and 0.1 cm yr⁻¹ for Dye 3, Crête, and Devon Island respectively. For the Greenland sites Dye 3 and Crête, the results are to be compared with a present-day surface elevation increase inferred from observations of the order of a few cm per yr (refs 15, 16). This shows that the mechanism discussed contributes

significantly to the present thickness change of the interior regions of the Greenland ice sheet. Furthermore, the progressive thinning of the basal soft ice layer implies that the potential ice thickness increase should persist over thousands of years to come, at Dye 3 with an almost unchanged rate in the nearest 5,000 years, and at Crête at a rate that will increase slightly in the nearest 10,000 years.

The hypothetical nature of these predicted thickness changes must be stressed. Actually, they may be overridden by the effects of mass balance and ice temperature variations caused by a changing climate. However, this study indicates that the different flow properties of Holocene and Wisconsinan ice (perhaps more generally the different properties of ice from glacial and interglacials?) must be considered when modelling the long term response of ice sheets to the glacial-interglacial climate cycles, on equal terms with the impact of mass balance and ice temperature variations. It is an interesting idea, that a possible alternating depositions of soft and hard ice through the glacial-interglacial cycles in itself will imply a potential cyclic behaviour in the thickness response of the ice sheets with an amplitude of the order of 100 m, and with minima/maxima occurring in the interior regions in late glacial, respectively interglacial to early glacial times. A likely cycle in accumulation rate (low values in cold glacial climates, high values in interglacials¹⁷) will imply a similar response, disregarding the delay of a few thousand years between extrema of the mass balance and the thickness response¹⁴. The response of the ice sheet to the glacial-interglacial temperature cycle is a matter of great complexity; the temperature variation of the deep layers of the ice sheet, which dominate the ice sheet response, not only depends on the temperature cycle at the surface, but also on the advective transport of cold downwards into the ice sheet, which in turn depends on a possible varying accumulation rate. A study of this complex problem for the Crête location on the Central Greenland ice sheet indicates that basal temperatures at this location reached their maxima/minima in late glacial and early glacial times respectively (W. S. B. Paterson and E. Waddington, manuscript in preparation). Since warm/cold basal ice implies soft/hard basal ice and consequently thin/thick ice sheets, it seems that the ice sheet response to the glacial temperature cycle in central Greenland enhances the direct response to accumulation rate changes and to the alternating soft-hard ice deposition. Therefore, the larger extent of the Greenland ice sheet in Wisconsinan time¹⁸ need not necessarily have implied greater ice thicknesses in the central regions as has often been assumed^{19,20}. On the contrary it is possible that the Greenland ice sheet in the (late) Wisconsinan was just as thin or even thinner than at present in spite of its greater geographical extent.

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Table 2 Modelled positions of the 10,000-yr age-horizons

Site	Relative depth	Height above bottom (m)
Dye 3	0.88	240
Crête	0.61	1,170
Devon Island	0.97	9

Seasonal variability and geochemical significance of organic matter in the River Ganges, Bangladesh

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Asian rivers, particularly those originating in the Himalayas, contribute significantly to the annual global sediment discharge from the land to the sea¹. Although some data are available on the inorganic geochemistry of these rivers²⁻⁶ little is known about their organic matter load^{7,8}. Over the past 4 years we have been studying the Ganges-Brahmaputra river system at locations in Bangladesh^{9,10} with sampling intervals of 4 to 6 weeks. Here we report on the seasonal variability of organic matter in the dissolved and suspended loads of the Ganges during 1981 as revealed by data on particulate and dissolved organic carbon (POC and DOC) and their constituent fractions such as sugars, amino acids and *n*-alkanes. Organic matter associated with peak sediment discharge (July to November) appears to be dominated by a biodegraded fraction, presumably also resulting from biogeochemical processes occurring in the ox-bow lakes in Bangladesh, and, as a consequence, are less susceptible to further decomposition upon reaching the marine environment. We suggest that rivers such as the Ganges with their high sediment load are significant in terms of land-derived organic carbon burial in marine sediments.

Within the framework of a program designed to assess the transport of carbon and minerals in major world rivers^{9,11,12} the Ganges (called Padma in Bangladesh) was monitored at Kazirghat, Bangladesh during 1981, with sampling intervals of 4 to 6 weeks. Details of the sampling location and sampling procedures are given in ref. 9. Water samples were filtered through precombusted (500 °C) glass fibre filters (Schleicher and Schüll, No. 6 vg) using a hand-operated filtration set. Organic carbon, sugars and amino acids were determined in the particulate and dissolved fractions according to methods described elsewhere¹³. Results are presented in Table 1.

More than 80% of the annual water discharge in the Ganges occurs between July and November of each year^{10,14}. This period also accounts for more than 80% of the annual sediment discharge¹⁰, with suspended matter concentrations up to 1,250 mg l⁻¹. POC contents were between 5 and 6% in March and May to 1 and 2% during periods of high sediment discharge and are comparable to those reported from the Indian stretch of the Ganges⁵. Such a decrease in POC contents with increase in sediment load is also apparent from data compiled for world rivers, in general⁸, and has been explained to be a consequence of dilution of organic matter by mineral matter. However, these compilations include very little information from the major

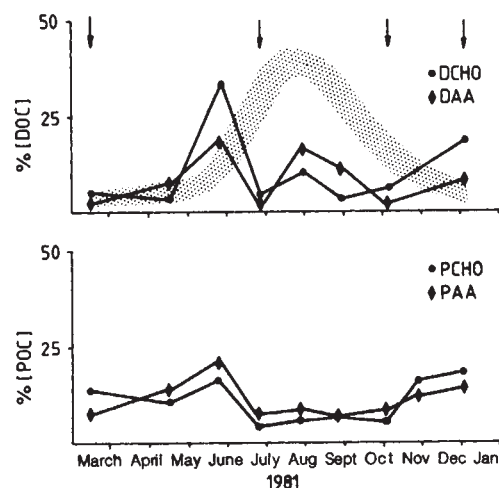


Fig. 1 Seasonal fluctuations in the percentage distribution of carbon contributed by sugars and amino acids to total dissolved (DCHO and DAA) and particulate (PCHO and PAA) organic carbon, in the Ganges, Bangladesh. Shaded area represents, schematically, the hydrographic curve. Arrows indicate samples analysed for *n*-alkanes.

South-East Asian rivers with their high sediment loads, and are based mainly on bulk organic carbon measurements from rivers of the temperate regions. Our data obtained from the analyses of constituent fractions such as sugars, amino acids and *n*-alkanes of POC transported by the Ganges, an Asian river with high sediment load, indicate that there are significant differences in the nature of POC transported during lean and high sediment discharge periods. Similar differences were also apparent in the DOC fraction. Its concentrations varied between 1.4 and 9.3 mg l⁻¹. The peak value was observed in July.

Sugars and amino acids are important food sources for microbial organisms and are rapidly recycled in the aquatic habitat¹⁵. Temporal variations in their contribution to the bulk DOC and POC have been used as indicators of biogeochemical processes in the aquatic sedimentary environment¹⁶⁻²⁰. Generally, microbially reworked (biodegraded) organic matter appears to contain less of these labile constituents. In the Ganges samples, concentrations of sugars varied between 46 and 1,672 µg l⁻¹ in the particulate and between 138 and 1,119 µg l⁻¹ in the dissolved fractions. The corresponding values for amino acids were between 24 and 2,395 µg l⁻¹, and between 156 and 638 µg l⁻¹, respectively. Significant here is the information revealed by the seasonal variation in the percentage of sugars and amino acids in the POC and DOC fractions (Fig. 1). The percentage of sugars and amino acids is highest for the June sample which corresponds to the initial stages of rising waters. After that throughout the entire section of the high sediment and water discharge period they are lower, indicating the biodegraded nature of organic matter transported. Additional evidence comes from the relatively higher contribution of individual amino acids such as β -alanine and γ -aminobutyric acid associated with the DOC and POC¹⁰. These amino acids are microbially mediated decarboxylation products of aspartic and glutamic acids, respectively^{16,20}. The relative distribution of these amino acids has been used as an indicator of microbially reworked organic matter²¹. Thus, there seems to be a significant difference in the nature of organic matter transported by the Ganges at low and high water and sediment discharge periods. Such differences may arise from inputs of organic matter from different sources and/or from biogeochemical processes.

To check whether inputs from different sources are reflected in more specific organic geochemical indicators, we determined the *n*-alkane distribution of some of the samples. For this, suspended matter on filters was subjected to multiple ultrasonic extraction with toluene/methanol (3:1 v/v). The saturated

Table 1 Dissolved and particulate organic carbon (DOC and POC), sugars (DCHO and PCHO) and amino acids (DAA and PAA) in the Ganges at Kazirghat, Bangladesh, 1981

Date	DOC (mg l ⁻¹)	DCHO (µg l ⁻¹)	DAA (µg l ⁻¹)	POC (%)	PCHO (µg l ⁻¹)	PAA (µg l ⁻¹)
30 March	3.8	373	321	5.71	46	24
3 May	2.3	190	399	6.11	151	174
23 June	1.3	1,119	619	1.26	338	419
24 July	9.3	1,042	507	1.15	1,672	2,395
30 August	1.6	430	638	0.98	1,265	1,917
27 September	1.4	138	397	0.73	585	862
1 November	2.9	484	156	1.69	305	351
29 November	—	—	—	2.04	260	187
3 January	2.7	1,215	552	1.36	110	83

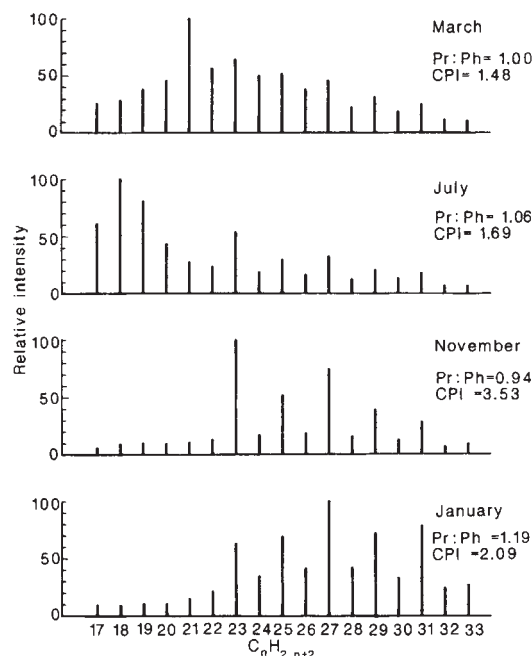


Fig. 2 Carbon number distribution of *n*-alkanes in POM of samples indicated in Fig. 1.

hydrocarbon fraction was separated from the total extract by column chromatography on silica gel using *n*-pentane as eluent. *n*-alkane spectra were run on a Carlo Erba high resolution gas chromatograph (GC).

Since small quantities of samples were available only a qualitative estimation of the variability in *n*-alkane spectra and the related sources could be made. They show marked variations in time (Fig. 2), especially in the July sample, where the contribution from algal organic matter is reflected by short chain (C_{17} , C_{18}) components and low CPI (carbon preference index) values (1.69). The dominance of *n*- C_{17} , *n*- C_{18} in *n*-alkane spectra has been suggested to result from algal inputs²². On the other hand, the bimodal distribution of *n*-alkanes in this sample with peaks also in the region of C_{23} – C_{27} suggests an additional hydrocarbon source. A complete shift in favour of odd-preferred long-chain molecules occurs during periods after July, resulting in increased CPIs (3.53) in the November sample. Sediments from tropical coastal swamps²³ have been reported to display comparable hydrocarbon distribution pattern. Neglecting the signals of algal inputs, organic matter transported by the Ganges during times of high sediment discharge (July to November) is strikingly similar, and differs significantly from the organic matter transported during low sediment discharge periods. The presence of *n*-alkanes in the samples may, at first sight, look inconsistent with the data presented above. However, biodegradation of *n*-alkanes proceeds at a much slower rate than sugars and amino acids. *n*-alkanes have been found in extracts of Recent sediments²⁴. Although our *n*-alkane spectra allow only source identification, their relative distribution pattern during high sediment discharge periods may indicate that biodegradation has also affected the *n*-alkanes below *n*- C_{23} (ref. 25).

Detailed chemical characterization of organic matter indicate significant temporal differences in the nature and source of organic matter. Biogeochemical processes in the ox-bow lakes in the Bangladesh sector of the Ganges drainage area may influence the nature of organic matter carried by the river²⁶. Bangladesh is literally pock-marked with such lakes. Based on our data, such processes appear to have a dominating influence on the nature of organic matter transported during rising and peak discharge (Fig. 3). The highest POC contents in the suspended load of the river are observed during the lean months (Fig. 3a). With an increasing water discharge, there is a dilution effect which continues until early overbank of the flood wave (Fig. 3b). The major mechanisms dictating the nature of organic

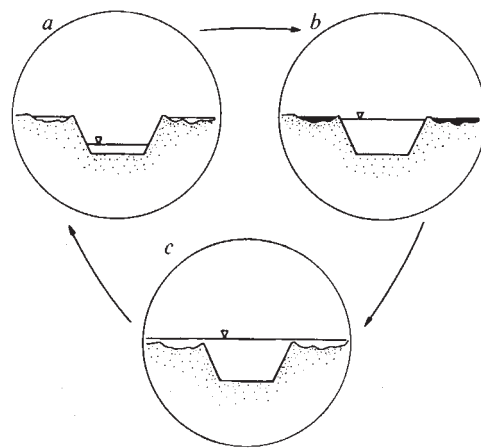


Fig. 3 Schematic diagram representing mechanisms controlling the nature of organic matter transported by the Ganges, Bangladesh. The dark areas in *b* depict the intense primary production occurring in the ox-bow lakes, ponds and topographic depressions such as Chalan Bill²⁶ during April–May. Anoxic conditions develop in some of them during lean months (Chowdhury, personal communication). With increasing water discharge organic matter from these areas is flushed out.

matter in periods following this appear to be (Fig. 3c); (1) During early overbank of the flood wave, organic matter produced in the ox-bow lakes enters the main channel through lake outflow. Chemical and biological measurements made on out-flowing waters from the lake indicate intense primary production during April–May²⁶. This would explain the high contribution of sugars and amino acids to both POC and DOC in the June sample. (2) Subsequently, with increasing water discharge, organic matter from the lakes are flushed out into the main channel because of thorough mixing of lake water and sediments with the river water. This is evidenced in the high DOC concentration and the bimodal distribution of *n*-alkanes (both algal and non-algal) in the POC in the July sample. On the other hand, the low contribution of sugars and amino acids to total POC and DOC and the distribution of β -alanine and γ -aminobutyric acid relative to aspartic and glutamic acids¹⁰ indicate a higher degree of microbial reworking of the organic matter. This aspect of the organic matter remains the same throughout the high-sediment and water discharge periods, but a shift in source from algal to non-algal is observed towards the end of the high-sediment discharge period as indicated by the dominance of *n*- C_{23} , *n*- C_{27} in the *n*-alkane spectra of POM (particulate organic matter). This non-algal source could originate through inputs from the reworked lake sediments and the soil organic matter.

The above data obtained from a major Asian river with high sediment load are of relevance to studies on global organic carbon transport from the land to the sea. One of the problems associated with the computation of the actual flux of carbon from the land to the sea is the uncertainty concerning the amount of organic matter being 'lost' in the estuarine and coastal marine environments. Calculations based on some of the European rivers indicate a loss of up to 70% (ref. 27). Other estimates also indicate the apparent 'instability' of riverine organic matter in the coastal sea²⁸. Organic geochemical tools such as those employed here should prove invaluable in assessing the role of labile versus stable organic matter in the rivers and its burial potential in marine sediments. Our data indicate that most of the organic matter associated with peak sediment discharge is significantly enriched in a fraction that is already biodegraded. Expressed differently, it is less susceptible to degradation on reaching the sea and, as a result, have a better chance of preservation in the marine geological record. Isolation and chemical characterization of specific organic molecules in river suspensions may thus prove to have great palaeoenvironmental significance.

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Dendroclimatological implications of isotope coherence in trees from Kashmir Valley, India

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Stable isotope ratios of hydrogen, carbon and oxygen in tree cellulose have recently been demonstrated¹⁻⁴ as potential palaeoclimatic indicators. A significant long-term objective of isotope dendroclimatology is the reconstruction of the annual climatic record at a given place. Earlier studies focused mainly on spatial correlations, selecting trees from climatically contrasting regions, rather than temporal correlations at a single site. Single-site correlation involves the problem of extracting a relatively weak climate-dependent isotope signal from a number of inherent non-climatic components (for example, isotope variations along the circumference of a single growth ring and inter- and intraspecies variations among trees growing in the same microclimate⁴⁻⁶). It is therefore important to ascertain the degree of coherence in the isotope records among different radial directions of a tree disk^{7,8} and different trees (same and different species) at a single site. Such coherences are demonstrated here based on δD , $\delta^{13}C$ and $\delta^{18}O$ measurements of individual rings of trees from Kashmir Valley, India.

Samples were collected⁹ in May 1980 in the form of disks from freshly felled 100-200-yr-old trees from a relatively open

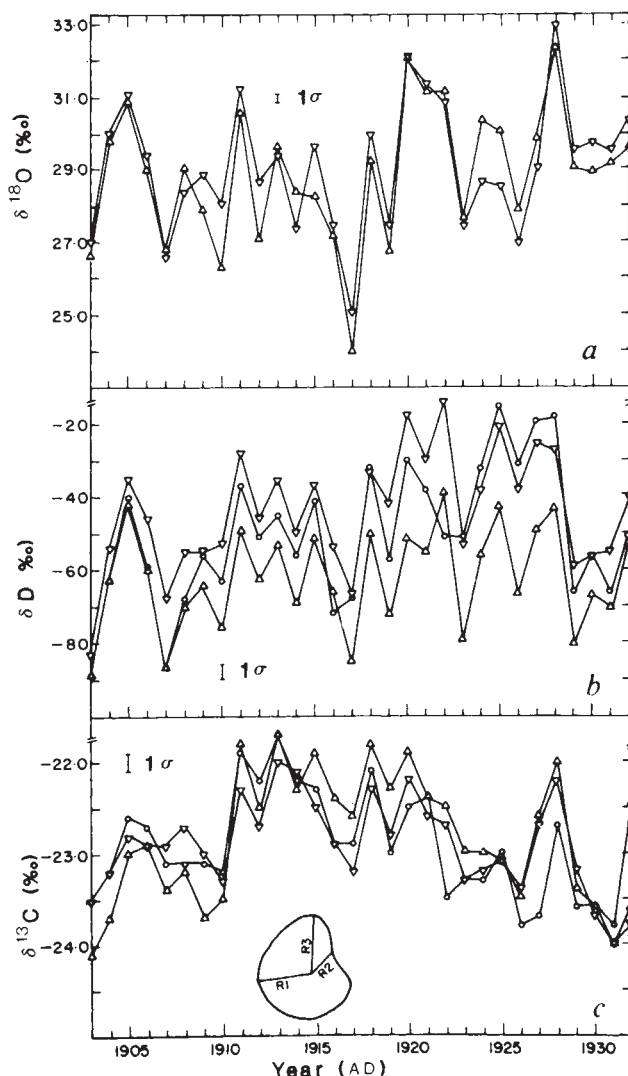


Fig. 1 a, $\delta^{18}O$ values of cellulose from individual rings along two radii R1 and R2 of tree AP2 (*Abies pindrow*). b, δD values and c, $\delta^{13}C$ values of nitrated cellulose from rings along three radii, R1, R2 and R3, of the same tree. Δ , Samples along R1; ∇ , samples along R2; $\circ$, samples along R3. Experimental uncertainty is given as 1σ (s.d.).

forest on a hilly terrain remote from permanent sources of water. The location was Gulmarg forest compartment 63 near Tangmarg ($74^{\circ}25' E$, $34^{\circ}04' N$, 2,650 m above sea level). Dates were assigned by ring counting and verified with the master chronology for this region (M. K. Hughes, personal communication). Thirty rings corresponding to the years AD 1903-32 (no missing or double rings) were chosen for analysis. They were wide enough (2-11 mm) to be sampled without cross-contamination. Standard procedures were used for the extraction of α -cellulose from wood¹⁰, its nitration¹¹ and gas preparation^{6,12,13}. α -Cellulose was used for $\delta^{18}O$ measurements and nitrated cellulose for δD and $\delta^{13}C$. Isotope ratios were measured using two VG Micromass 602D mass spectrometers with an overall precision of $\pm 2\%$ for δD , $\pm 0.1\%$ for $\delta^{13}C$ and $\pm 0.2\%$ for $\delta^{18}O$. Values for δD and $\delta^{18}O$ are expressed relative to SMOW¹⁴ and $\delta^{13}C$ to PDB¹⁵. The details of the experimental procedures are reported elsewhere¹⁶.

The first experiment determined the extent of coherence of the isotope signal among the different radial directions of a silver fir tree (*Abies pindrow*), AP2. This specimen was selected for its asymmetrical growth, which was likely to introduce an unfavourably large dispersion in the data. δD and $\delta^{13}C$ were

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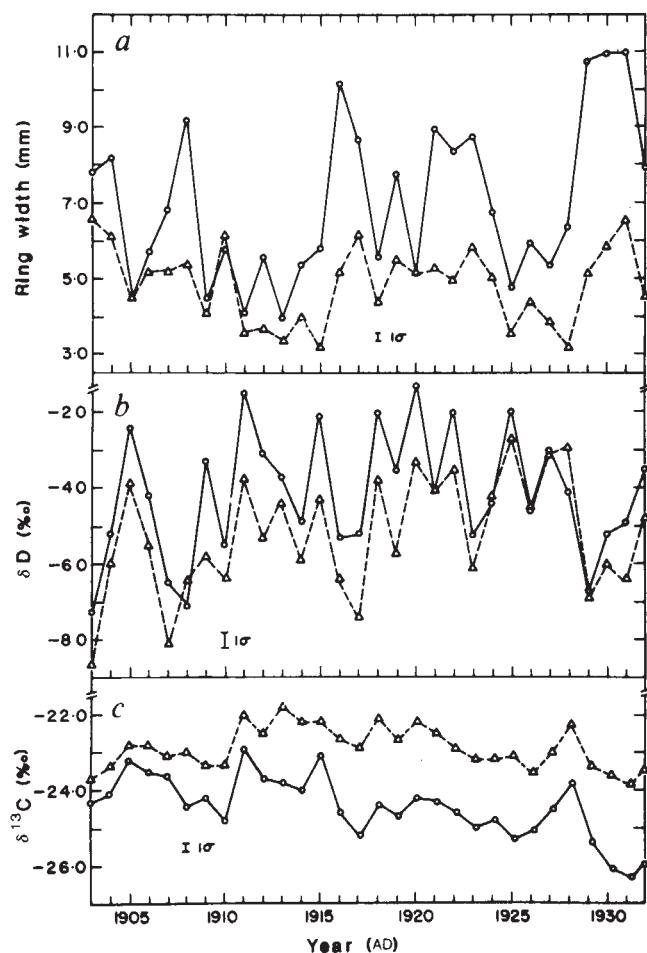


Fig. 2 a, Ring width, b, δD and c, $\delta^{13}C$ values of individual rings from AP2 and AP3 (*A. pindrow*). Ring widths for both trees are average values of measurements along different radial directions. δD and $\delta^{13}C$ of nitrated cellulose are means of three radial measurements in the case of AP2. $\circ$, Samples from AP3; $\triangle$, samples from AP2.

measured along three radii (R1 ~ 32 cm, R2 ~ 20 cm and R3 ~ 35 cm) and $\delta^{18}O$ along two (R1 and R2).

The results (Fig. 1) show that although the absolute δ values do not agree among the different radii, the trends are quite similar. The maximum difference between any two radii was 33% for δD , 1.8% for $\delta^{18}O$ and 1.3% for $\delta^{13}C$. Although in some of the rings the δ values agreed within experimental error at least among two radii, in most cases the extent of the differences varied. Thus, the intra-ring isotope variability seems to be unsystematic and its absence in a single ring or a few arbitrarily selected rings does not necessarily imply the absence of intra-ring variability in the tree, as some workers¹⁷ have presumed.

Shading and consequent changes in the CO_2 assimilation rate have been suggested¹⁸ to cause variation in intra-ring $\delta^{13}C$, but here free-standing trees, exposed to similar light levels, exhibited circumferential variation in all three isotopes. Therefore, we propose instead that the asymmetrical growth of a tree results from lateral redistribution or asymmetrical production of a growth-regulating hormone, a growth inhibitor or an auxin-destroying enzyme¹⁹. Depending on how and when such a redistribution or production takes place, some part of the cambium may be active during a growing season while another is less active or even dormant. Consequently, the cells growing at the various spots along the circumference of a growth ring may use food material produced at slightly different times of the growing season. The isotope concentrations at these different parts are values integrated over the entire growing season. The integrating period, if slightly different for these spots, could lead to circumferential isotope variability. This process may vary

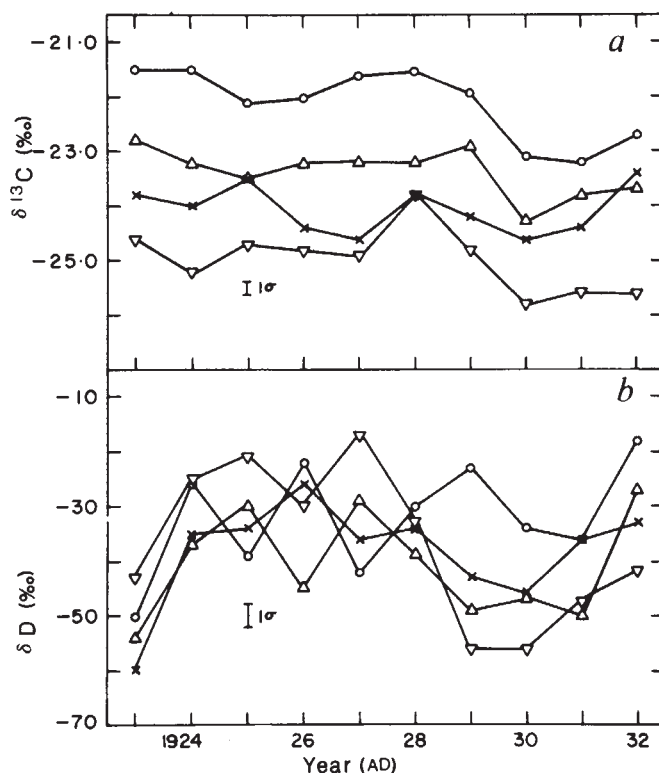


Fig. 3 a, $\delta^{13}C$ and b, δD values of nitrated cellulose from early wood part of individual rings from four trees. $\circ$, Samples from CD1 (*Cedrus deodara*); $\triangle$, samples from AP2 (*Abies pindrow*); $\times$, samples from PW3 (*Pinus wallichiana*); ∇ , samples from AP3 (*Abies pindrow*).

from year to year depending on the hormone activity (perhaps controlled by crown vigour), resulting in a non-uniform intra-ring variation for the different years.

The 30-yr data for each of the isotopes along each of the radii were standardized by dividing the deviations from the mean of the respective set by the standard deviation of the set. Thus, the data sets are transformed to have means of zero and standard deviations of unity, somewhat similar to the standard tree-ring indices. For each of the δD , $\delta^{18}O$ and $\delta^{13}C$ records, the variance common to the different radii was then calculated using a procedure for analysis of variance outlined by Fritts¹⁹. This common variance, interpreted as the common signal among the different radii, was ~95% for δD and $\delta^{18}O$ and ~89% for $\delta^{13}C$. The intra-ring isotope variability in this case thus represents a 'noise' of ~5% in δD and $\delta^{18}O$ and ~11% in $\delta^{13}C$ records. This noise is probably even less in the case of trees where the hormone distribution may have been more uniform along the circumference of the cambium, leading to a more symmetrical growth.

Our second experiment determined the coherence of the δD and $\delta^{13}C$ signals in two silver fir trees, AP2 and AP3, which grew ~10 m apart. Thirty individual rings taken from a single radial strip of AP3, which had a more symmetrical growth than AP2, were analysed. Figure 2 shows the results, together with the ring-width variations (the values for AP2 are the means of the values along the three radii, discussed earlier). The δD curves cross each other at certain points while the $\delta^{13}C$ curves are quite distinct. $\delta^{13}C$ values of AP2 are generally higher than those of AP3 by a mean of $1.6 \pm 0.6\%$. This difference could be due to intra-ring variation along the circumference of AP3, where only one radial section was analysed.

The δD and $\delta^{13}C$ signals are indeed coherent. The common signal between the two trees was calculated (after standardizing the AP3 data as explained previously) to be ~92% for δD and ~83% for $\delta^{13}C$. Allowing for the 'noise' due to the intra-ring isotope variability found earlier, this coherence is quite good.

The third experiment was designed to study the species effect on the coherence of the isotope signal from four trees growing within a distance of ~50 m. Two were firs (*A. pindrow*, AP2 and AP3), one a deodar (*Cedrus deodara*, CD1) and the other a pine (*Pinus wallichiana*, PW3). These are the major species of conifers growing at Gulmarg.

Earlier studies have shown that large differences exist between the δ values for early and late wood^{6,20,21}. To avoid possible interference from differing amounts of early wood and late wood in the three species, only early wood was therefore analysed. A small portion of the early wood adjacent to the late wood was left out, so as to avoid contamination. δD and $\delta^{13}C$ values were measured in cellulose nitrate from 10 rings of each tree corresponding to the interval AD 1923–32. These rings were broad enough (~6.2 mm) to facilitate easy sampling of relatively pure early wood. The coherence (as defined earlier) in the isotopic signal among the four trees was found to be ~79% for δD and ~84% for $\delta^{13}C$ (Fig. 3). The coherence in the δD signal among different species of trees was less than that between two trees of the same species. This may be partly due to differences in the temperature dependence of the biochemical fractionation factor for deuterium and in the growing seasons for the different species¹⁷.

To check whether additional noise had been introduced through the sampling of a non-constant percentage of each annual ring (in the form of early wood), the intra-ring variation in early wood alone was investigated. δD and $\delta^{13}C$ values were measured in nine segments of the early-wood part of the 1923 ring of PW3, and showed large asymmetry—values ranging from -55 to -67‰ for δD and from -23.7 to -24.5‰ for $\delta^{13}C$ —indicating that the sampling procedure is unlikely to have introduced significant additional noise to the signal. The mean difference between the $\delta^{13}C$ values of whole-ring cellulose of AP2 and AP3 (1.6‰) was still maintained in the early-wood measurements, lending additional support to this argument.

The isotope signals from different radial sections of an *A. pindrow* disk from the Gulmarg site are highly coherent. So also are the isotope signals from two trees of the same species and four trees of different species from this site. The presence of such a common pattern of variability in trees²² in a single region indicates the possibility of obtaining climatic information on a temporal scale from the isotope systematics in tree cellulose from a single site. The correlation of the isotope record in Kashmir with instrumentally measured local climatic parameters will be described elsewhere²³.

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Optimization strategies gleaned from biological evolution

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Several problems, in particular the 'travelling salesman' problem¹ wherein one seeks the shortest route encompassing a randomly distributed group of cities, have been optimized by repeated random alteration (mutation) of a trial solution followed by selection of the cheaper (fitter) solution. Most non-trivial problems have complicated fitness functions, and optimization tends to become stuck in local fitness maxima. A recently introduced strategy to escape (simulated annealing) involves accepting unfavourable mutations with finite probability^{1–3}. Independently, there has been interest in genetic strategies which overcome the problem of fitness maxima in biological evolution^{4–6}, and several authors have applied biological elements to optimization^{7,8}. Here we use computer algorithms to investigate new strategies for the 64-city travelling salesman problem, which combine conventional optimization or 'quenching' with biological elements, namely having a population of trial solutions, helping weaker individuals to survive, and an analogue of sexual crossing-over of genes. The new strategies were faster and gave better results than simulated annealing.

During the course of evolution, slowly evolving genes would have been overtaken by genes with better evolutionary strategies. Consequently, one expects highly efficient strategies in modern flora and fauna. Our purpose here is not to model biological evolution, but to attempt to glean useful optimization strategies from it. Biological strategies (reviewed in refs 4–6) include: point DNA mutations; duplication and deletion mutations in sexual crossing-over; redundancy (over 80% of human DNA has no apparent function); sexual mixing of genes; genetic drift; and jumping genes.

There are mechanisms in sexual species which reduce evolutionary competition between genes. For example, unfavourable genes are helped to survive if they are recessive in a diploid organism or if there are multiple copies of genes. One consequence is a wide diversity of individuals, as was observed by Darwin⁹. Another consequence is to encourage division into species if the genetic burden⁴ becomes high.

A lineage which helps weak individuals to survive can have a long-term evolutionary advantage. We illustrate this with a greatly simplified example. Suppose there is a probability F (<1) that a species will encounter some sort of catastrophe or will reach a local fitness maximum and stop evolving. If the lineage divides into n independent species (some of which will, of course, be weaker than the others at any given time), the probability that all of them encounter catastrophe or stagnate is smaller, F^n . Weighing against this advantage, resources must be shared so that each species has fewer individuals and evolves more slowly.

Evidence from several fields indicate that on balance there can be a net advantage to this type of diversification. In the fossil record for lungfish, an association was drawn¹⁰ between rapid evolution and rapid speciation, and conversely between stagnation and a small number of lineages. Wide diversity has recently been observed to develop^{11,12} in the DNA of lymphocyte cells, which need to evolve rapidly to improve the body's immune response. An explanation similar to the above has been proposed to account for the diversity of human language and culture¹³. To accelerate scientific progress, Feyereabend¹⁴ recommends, amongst other things, 'proliferation of theories'; and Kuhn¹⁵ has observed periods of stagnation in scientific progress associated with reduced diversity of theories when there was one encompassing theory (such as, phlogiston).

Our experimental system was the well-studied 'travelling salesman' problem¹, where the shortest route was sought which visited 64 cities randomly placed in a square, returning to the

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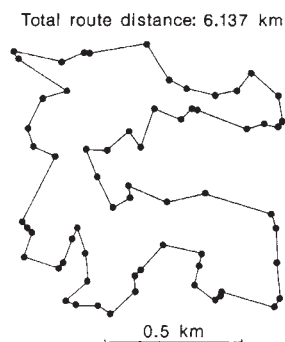


Fig. 1 Map showing the cities visited by the travelling salesman, and the shortest route ever found.

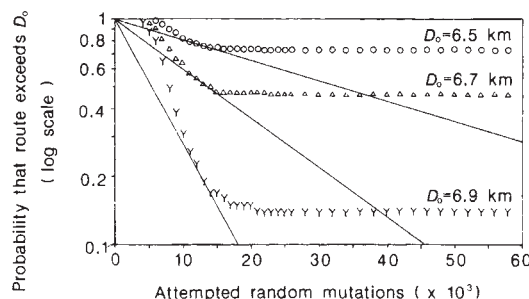


Fig. 2 Data from 100 optimizations using the simplest strategy: quenching by random attempted mutations, accepting only favourable ones. The proportion of optimizations whose route was longer than certain target distances, is plotted against number of attempted mutations. The straight lines represent the optimum speciation strategy (see text).

first city. The roads between cities were straight. It was not feasible to try all 631/2 different possible routes, so an optimization technique had to be used. Results are presented below for the map shown in Fig. 1, which also shows the shortest route found.

In the simplest optimization scheme, a random starting route was chosen. Repeatedly, a section of the route was chosen at random, and the order of visiting the cities in the section was reversed if this shortened the route. At no stage was an unfavourable change accepted, so that this scheme may be called 'zero temperature' simulated annealing, or 'quenching'. One hundred separate optimizations were performed, and Fig. 2 shows the proportion $F(t)$ for which the routes were longer than 6.5 km, 6.7 km and 6.9 km after t attempted mutations.

This optimization may be improved by a strategy akin to speciation. If computer time were shared between n independent solutions and the best solution selected at the end, the new corresponding curves would be (see discussion of speciation above):

$$F_n(t) = (F(t/n))^n \quad (1)$$

Note that this equation takes into account that central processor unit (CPU) time must be shared between trial solutions so that each evolves n times more slowly. Several authors have considered optimizations using massively parallel processing machines^{16,17}; further improvement may be obtained using such hardware but this is not discussed here.

The points $F_1(t)$, $F_2(2t)$, $F_3(3t)$ and so on may be shown from equation (1) to lie on a straight line on the semi-logarithmic scale of Fig. 2. The straight lines marked, which are all tangential to the plotted points at approximately $T = 13,000$ attempted mutations, represent the optimum speciation strategy. For example, if computer time were available for $2T$ attempted mutations the best strategy would be to divide time between two independent trial solutions and select the best. The expected performance may be read off from the straight lines in Fig. 2.

Figure 3 is a direct comparison of several different optimization strategies. One hundred separate optimizations were performed in each case, and Fig. 3 shows the mean distance obtained, plotted as a function of the mean computer time used on an IBM 3081D. Curve A is for simple quenching described above; whilst in curve B the strategy was to divide computer time equally between two independent trial solutions and select the better solution. As predicted, this latter strategy gave improved results at long times.

In order to evaluate the effect of competition between trial solutions (curve C), computer time was shared equally between two independent solutions. Every $1,000 \times 2$ attempted random mutations, the better of the two solutions was copied over, obliterating the weaker one. Surprisingly, this competitive strategy was far worse than for the noncompetitive one (curve B). Our interpretation is that success at an early stage in the

optimization was not closely correlated with success later on. Indeed, the performance was worse than for the noncompetitive strategy because diversity was reduced.

We conclude that if a strategy is to favour the more successful solutions it should be designed not to severely reduce diversity. A clue to how to do this is provided by sexual species, where genes can be swapped during mating and competition can be effectively on a smaller scale, between genes rather than between individuals. In the case of the travelling salesman, pictorially speaking, suppose that one salesman has developed a good route around Scotland whilst another has independently found a good route around Wales; a good strategy might involve competition between these subroutes and a method of mating or 'crossing-over' so that the successful subroutes may be combined. Simple 'mating' processes have been employed in previous work on a different model¹⁸.

The strategy adopted was as follows (Fig. 3, curve D). Computer time was shared equally between two independent trial solutions, as above. Every $1,000 \times 2$ attempted random mutations, the two solutions were compared and if possible a point was found where different subroutes visited the same cities. For example, suppose that one route visited cities ABCDEFG respectively, whilst the other visited cities GCADBFE. These routes contain two different subroutes for cities ABCD. The shorter subroute was then copied over, obliterating the longer one. In the example, suppose that distance $AB + BC + CD$ was smaller than $CA + AD + DB$; the shorter subroute was ABCD and the process would yield new routes ABCDEFG (unchanged) and GABCDFE. In the actual optimizations it was found best to search for subroutes of length between 8 and 32 (at least one was almost always found); and matings were attempted so infrequently because an appreciable amount of CPU time was spent in searching for possible crossing-over points (the time spent was of the order of LM if there were L cities and M different lengths to be compared).

This strategy gave an improvement at long times (see curve D). A further improvement was obtained as follows. Instead of attempting mutations randomly, a systematic search was made for favourable mutations. This could be extremely fast, both because random numbers need not be generated and because intermediate results relating to several attempted mutations could be calculated once only. The routes were optimized or 'quenched' in this way until all subroute reversals would be unfavourable. A crossing-over was then attempted as described above, and the altered route was quenched again before another mating. Curve E shows the improvement obtained. Further improvement was possible (curve F) by having 12 individuals rather than two. The individuals were paired randomly so that six matings could be attempted. The six altered individuals were then quenched before another set of attempted matings. We presume that performance was better because there was a wider gene pool from which to choose subroutes.

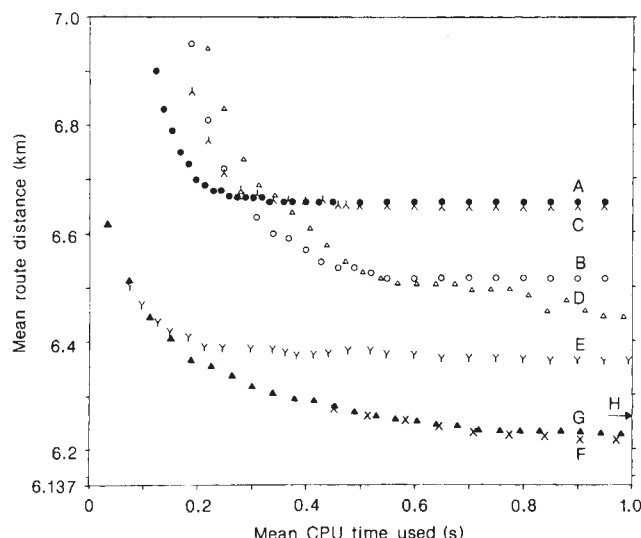


Fig. 3 Direct comparison of eight different optimization strategies, showing the mean route distance, plotted against mean computer time used on IBM 3081D. In each case, data were gathered from 100 separate optimizations. A, 'Quenching' by random attempted mutations (from the same data as Fig. 2). B, The best of two independent quenches. C, Competition between two quenches. D, Mating between two quenches. E, Mating between two quenches by systematic search. F, Mating between 12 quenches by systematic search. G, Best of n independent quenches by systematic search, $n = 1, 2, 3, \dots, 26$. H, Simulated annealing; 20,000 random attempted mutations per temperature; statistics from 20 optimizations only. Mean, 5.6 s.

Results almost as good as curve F were obtained by applying the systematic quench to a population of N independent trial solutions, and selecting the best solution. Curve G shows the mean performance for $n = 1, 2, 3$, up to 26 (compare curve B for a random quench and a population of 2). We believe it to be coincidental that curves F and G are close to one another, because the algorithms were different, and as a typical optimization in G took about 2–3 times as long as in F where the subroutes were already optimized. This is of importance because they may give different performances with larger problems.

For comparison, a set of simulated annealing optimizations were performed using the same map. At each temperature T , there were N random route reversals attempted: if a route reversal cost D km, it was accepted with probability $1 (D < 0)$ or with probability $\exp(-D/T) (D > 0)$. T took values $1/2, 1/4, 1/8, \dots, 1/1,024$ km. At $N = 10,000$ it took a mean 2.9 s to achieve a mean distance of 6.32 km; whilst at $N = 20,000$ the results were 5.9 s and 6.26 km (see arrow on Fig. 3). This strategy was worse than both F and G in final performance and CPU time used.

To conclude, biological strategies—having a large population, helping weaker individuals to survive, and competing at a gene level rather than at the level of individuals—were adapted successfully to the 'travelling salesman' problems. Combined with simple quenching they gave an improvement over conventional simulated annealing for the 64-city problem. It would be interesting, but would require extensive computing power, to gather good statistics and compare strategies for larger problems. The new strategies may be ideally suited for parallel processing hardware.

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Postmortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction

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Since its introduction in 1977¹, stable isotope analysis of bone collagen has been widely used to reconstruct aspects of prehistoric human and animal diets^{2–11}. This method of dietary analysis is based on two well-established observations, and on an assumption that has never been tested. The first observation is that bone collagen $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios reflect the corresponding isotope ratio of an animal's diet^{1–5,12}. The second is that groups of foods have characteristically different $^{13}\text{C}/^{12}\text{C}$ and/or $^{15}\text{N}/^{14}\text{N}$ ratios^{13,14}. Taken together, the two observations indicate that the isotope ratios of collagen in the bones of a living animal reflect the amounts of these groups of foods that the animal ate. Thus, it has been possible to use fresh bone collagen $^{13}\text{C}/^{12}\text{C}$ ratios to determine the relative consumption of C_3 and C_4 plants^{15–17}, while $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios have been used to distinguish between the use of marine and terrestrial foods¹⁴. The $^{15}\text{N}/^{14}\text{N}$ ratios of fresh bone collagen probably also reflect the use of leguminous and non-leguminous plants as food⁵, but this has not yet been demonstrated. Prehistoric consumption of these same groups of foods has been reconstructed from isotope ratios of collagen extracted from fossil bone^{1–11}. Implicit in the application of the isotopic method to prehistoric material is the assumption that bone collagen isotope ratios have not been modified by postmortem processes. Here I present the first examination of the validity of this assumption. The results show that postmortem alteration of bone collagen isotope ratios does occur, but that it is possible to identify prehistoric bones whose collagen has not undergone such alteration.

Bones of mammalian marine carnivores and terrestrial herbivores were obtained from the archaeological sites listed in Table 1. These particular types of animals were chosen because the isotope ratios in collagen from the bones of modern mammals possessing the same feeding habits have been well characterized¹⁴. The bones range in age from a few hundred to several thousand years old. The sites represent a variety of depositional environments, ranging from the extremely dry desert along the Peruvian coast, through coastal and interior sites of intermediate wetness in Florida and California, to a bog deposit in Denmark. The concentrations and isotopic compositions of carbon and nitrogen in collagen prepared from the bones, determined as described previously¹⁴, are also given in Table 1.

Figure 1 is a plot of nitrogen isotope ratios against carbon isotope ratios for all the prehistoric samples except the marine carnivores from sites in Florida, which are treated separately

Table 1 Concentrations and isotopic compositions of carbon and nitrogen in collagen from bones of animals excavated from various archaeological sites

Taxonomy	Site	Age (yr BP)	C/N	$\delta^{15}\text{N}$ (‰)†	$\delta^{13}\text{C}$ (‰)†	Taxonomy	Site	Age (yr BP)	C/N	$\delta^{15}\text{N}$ (‰)†	$\delta^{13}\text{C}$ (‰)†
Terrestrial herbivores						Marine carnivores—vertebrate eaters					
1013 <i>Cervus elaphus</i>	MD	6,600–5,700	3.3	+2.7	–21.4	1019 <i>Halicholrus grypus</i>	MD	6,600–5,700	3.6	+12.6	–12.0
1014 <i>C. elaphus</i>	MD	6,600–5,700	3.8	+3.0	–22.5	795 <i>Monachus tropicalis</i>	WF	1,500–1,250	3.4	+11.6	–10.1
1015 <i>C. elaphus</i>	MD	6,600–5,700	3.2	+4.1	–22.3	796 <i>M. tropicalis</i>	WF	1,500–1,250	3.4	+10.2	–7.8
1016 <i>C. elaphus</i>	MD	6,600–5,700	3.7	+3.9	–22.3	797 <i>M. tropicalis</i>	WF	1,500–1,250	3.3	+10.8	–9.9
1017 <i>C. elaphus</i>	MD	6,600–5,700	3.4	+5.6	–23.5	780 <i>Tursiops truncatus</i>	GF	1,500–600	3.2	+9.7	–4.0
904 <i>Odocoileus hemionus</i>	SC	850–200	3.2	+4.8	–20.4	782 Delphinidae/ Physeteridae	PF	4,000–1,000	3.4	+8.4	–11.0
905* <i>O. hemionus</i>	RC	5,500–850	7.2	+12.3	–24.5	783 Delphinidae/ Physeteridae	PF	4,000–1,000	3.3	+10.6	–9.7
906 <i>O. hemionus</i>	MC	5,500–850	3.3	+4.6	–19.7	794 Delphinidae/ Physeteridae	WF	1,500–1,250	3.3	+10.5	–8.0
907 <i>O. hemionus</i>	UC	850–200	3.2	+4.5	–20.2	802 Delphinidae/ Physeteridae	MF	600–300	3.6	+7.1	–15.0
910 <i>O. hemionus</i>	AC	850–200	3.2	+4.3	–20.2	803 Delphinidae/ Physeteridae	MF	600–300	3.3	+10.2	–9.4
911 <i>O. hemionus</i>	PC	3,400–200	3.3	+4.9	–20.7	787 Otariidae	CP	2,100–1,900	3.2	+17.2	–10.8
914 <i>O. hemionus</i>	GC	5,500–3,400	>5	+4.0	–23.3	788 Otariidae	CP	1,000–600	3.2	+19.5	–12.8
925* <i>O. hemionus</i>	SC	850–200	3.3	+4.9	–20.2	869 Otariidae	LP	7,000–4,600	9.8	+21.9	–18.5
926 <i>O. hemionus</i>	SC	850–200	3.2	+5.1	–20.4	875 Otariidae	LP	7,000–4,600	3.3	+17.3	–11.5
927 <i>O. hemionus</i>	SC	850–200	3.2	+6.8	–19.7	882* Otariidae	LP	7,000–4,600	10.7	+13.0	–18.2
928 <i>O. hemionus</i>	SC	850–200	3.3	+4.4	–19.3	884 Otariidae	LP	7,000–4,600	<2	+11.9	–22.2
948 <i>O. hemionus</i>	MC	5,500–850	3.3	+5.6	–19.8	886 Otariidae	LP	7,000–4,600	7.2	+17.3	–18.6
949 <i>O. hemionus</i>	MC	5,500–850	3.2	+5.5	–19.2	892 Otariidae	LP	7,000–4,600	4.3	+14.5	–19.3
960 <i>O. hemionus</i>	UC	850–200	3.2	+4.3	–19.9	894 Otariidae	LP	7,000–4,600	7.6	+21.3	–17.8
965 <i>O. hemionus</i>	LC	5,500–850	3.3	+4.4	–19.8	896 Otariidae	LP	7,000–4,600	>7	+14.1	–14.1
966 <i>O. hemionus</i>	LC	5,500–850	3.3	+3.6	–19.7	920 Otariidae	SC	850–200	3.2	+17.0	–13.1
967 <i>O. hemionus</i>	LC	5,500–850	3.8	+1.4	–20.5	922 Otariidae	SC	850–200	3.2	+17.3	–13.5
968 <i>O. hemionus</i>	LC	5,500–850	3.4	+3.8	–20.0	923 Otariidae	SC	850–200	3.5	+17.1	–13.0
977 <i>O. hemionus</i>	SC	>200	3.3	+5.6	–19.7	924 Otariidae	SC	850–200	3.2	+16.2	–13.2
980 <i>O. hemionus</i>	AC	850–200	3.3	+6.1	–18.8	940 Otariidae	RC	5,500–850	3.3	+19.6	–12.3
981 <i>O. hemionus</i>	AC	850–200	3.2	+5.9	–19.0	943 Otariidae	MC	5,500–850	3.2	+18.8	–12.2
982 <i>O. hemionus</i>	AC	850–200	3.2	+5.8	–19.0	944 Otariidae	MC	5,500–850	>8	+0.2	–22.4
998 <i>O. hemionus</i>	PC	3,400–200	3.2	+4.6	–20.0	945 Otariidae	MC	5,500–850	3.2	+18.2	–12.1
999 <i>O. hemionus</i>	PC	3,400–200	>6	+6.8	–22.7	946 Otariidae	MC	5,500–850	3.8	+17.7	–15.2
781 <i>Odocoileus virginianus</i>	GF	1,500–600	3.2	+5.3	–20.3	951 Otariidae	UC	850–200	3.2	+17.5	–13.4
784 <i>O. virginianus</i>	PF	4,000–1,000	3.3	+4.6	–20.4	952 Otariidae	UC	850–200	3.2	+17.7	–11.5
785 <i>O. virginianus</i>	PF	4,000–1,000	3.3	+4.6	–20.6	962 Otariidae	LC	5,500–850	3.4	+19.3	–12.9
786 <i>O. virginianus</i>	PF	4,000–1,000	3.3	+5.5	–20.5	963 Otariidae	LC	5,500–850	3.5	+16.7	–13.8
798 <i>O. virginianus</i>	WF	1,500–1,250	3.3	+6.1	–18.5	964 Otariidae	LC	5,500–850	>7	+8.1	–19.5
799 <i>O. virginianus</i>	WF	1,500–1,250	3.2	+5.3	–19.2	976 Otariidae	XC	>200	3.3	+16.6	–13.2
800 <i>O. virginianus</i>	WF	1,500–1,250	3.2	+5.5	–18.6	983 Otariidae	AC	850–200	3.2	+17.4	–13.3
801 <i>O. virginianus</i>	WF	1,500–1,250	3.4	+4.0	–18.0	985 Otariidae	AC	850–200	3.2	+19.0	–13.0
804 <i>O. virginianus</i>	MF	600–300	3.2	+4.8	–20.0	986 Otariidae	AC	850–200	3.3	+16.9	–13.2
805 <i>O. virginianus</i>	MF	600–300	3.3	+3.9	–20.5	1018 Phocidae	MD	6,600–5,700	8.3	+10.3	–25.2
806 <i>O. virginianus</i>	MF	600–300	3.4	+5.3	–20.4	1020 Phocidae	MD	6,600–5,700	3.3	+15.1	–11.2
807 <i>O. virginianus</i>	MF	600–300	3.3	+3.8	–20.6	1021 Phocidae	MD	6,600–5,700	3.3	+15.0	–11.5
808 <i>O. virginianus</i>	MF	600–300	3.3	+4.3	–20.0	915* Phocidae/Otariidae	GC	5,500–3,400	>5	+3.1	–21.9
901 <i>O. virginianus</i>	CP	410±65	3.2	+9.5	–20.6	917 Phocidae/Otariidae	GC	5,500–3,400	>6	+7.2	–20.9
871 Cervidae	LP	7,000–4,600	6.5	+25.7	–17.6	950 Phocidae/Otariidae	UC	850–200	3.2	+18.4	–13.5
881 Cervidae	LP	7,000–4,600	3.2	+8.4	–19.0	984 Phocidae/Otariidae	AC	850–200	3.2	+19.1	–12.3
887 Cervidae	LP	7,000–4,600	3.4	+8.8	–19.0	987 Phocidae/Otariidae	AC	850–200	3.2	+18.0	–12.9
						989 Phocidae/Otariidae	PC	3,400–200	3.2	+17.5	–11.7
						991 Phocidae/Otariidae	PC	3,400–200	3.2	+19.4	–12.9
						992 Phocidae/Otariidae	PC	3,400–200	3.2	+18.8	–13.1
Marine carnivores—invertebrate or vertebrate eaters											
921 Cetacea	SC	850–200	3.2	+14.7	–12.6						
938 Cetacea	RC	5,500–850	>8	+9.5	–21.0						
975 Cetacea	XC	>200	3.7	+12.0	–14.5						
988 Cetacea	PC	3,400–200	>7	+54.7	–18.7						
990 Cetacea	PC	3,400–200	6.4	+18.9	–18.8						
939* Cetacea/Pinnipedia	RC	5,500–850	0.9	–0.9	–21.5						
953 Cetacea/Pinnipedia	UC	850–200	3.2	+12.4	–14.1						
954 Cetacea/Pinnipedia	UC	850–200	3.3	+18.0	–12.1						

The ages of the bones as well as the feeding behaviours of the animals^{14,21} are indicated. In some cases, C/N ratios could only be approximated because of the small amounts of gas generated during combustion of the sample. The site names and their locations are abbreviated as follows: AC, Alamo Pintado, California; CP, Chilca, Peru; GC, unnamed site on Goleta Slough, California; GF, Granada, Florida; LC, Lake Cachuma, California; LP, La Paloma, Peru; MC, Mescalito, California; MD, Maglemosegaard, Denmark; MF, McLarty, Florida; PC, Prisoners' Harbor, California; PF, Palmer, Florida; RC, Rincon, California; SC, Saxtilil, California; UC, unnamed site on Santa Cruz Island, California; WF, Wightman, Florida; XC, Little Pine Mountain, California.

* Sample burned prehistorically.

† Isotope ratios are expressed in the δ notation, where

$$\delta^*X = \left[\frac{(*X/X)_{\text{sample}}}{(*X/X)_{\text{standard}}} - 1 \right] \times 1,000\%$$

For carbon, $*X/X$ is $^{13}\text{C}/^{12}\text{C}$ and the standard is the PDB carbonate, while for nitrogen, $*X/X$ is $^{15}\text{N}/^{14}\text{N}$ and the standard is atmospheric (air) nitrogen. The precisions of the isotope measurements reported here are $\pm 0.2\%$. Elemental concentrations are reported as atomic C/N ratios. The precisions of the C/N ratios are ± 0.1 .

below. For comparison, the distribution of bone collagen isotope ratios of modern animals possessing various feeding specializations¹⁴ is also shown. Prehistoric collagen samples with C/N ratios between 2.9 and 3.6, the range that encompasses the values for collagen in 172 fresh bones from 69 animal species and 40 bones from modern humans (refs 5, 11, 14, 18–20 and M. J. Schoeninger and M.J.DeN., unpublished data), have isotope ratios that fall within the ranges observed in modern animals of the same feeding persuasion. On the other hand, some of the prehistoric collagen samples with C/N ratios outside the 2.9–3.6

range have $^{13}\text{C}/^{12}\text{C}$ and/or $^{15}\text{N}/^{14}\text{N}$ ratios that fall substantially outside the ranges exhibited by their modern counterparts.

Marine carnivores from the Florida sites are not included in Fig. 1 because they, unlike the other marine mammals included in this study, fed in environments in which there were substantial amounts of freshwater- and/or coral reef-based biomass available. The bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for modern animals that feed in these environments are different from those of animals feeding in the rest of the ocean¹⁴. The prehistoric collagen samples from the Floridian marine carnivores, all of

which have C/N ratios between 3.2 and 3.5, have $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values spanning the same ranges as bone collagen from modern animals feeding in similar environments¹⁴.

The observed postmortem alteration of bone collagen isotope ratios is not a function of the age of a sample, as some of the oldest samples retain their *in vivo* isotope ratios (samples 875, 881, 887, 1013, 1015, 1017, 1020 and 1021). The processes responsible for postmortem alteration do not affect all bones from a particular depositional environment. For example, some bones excavated at La Paloma, Peru have not suffered isotopic alteration whereas others of comparable age have (compare 875, 881 and 887 with 869, 871, 884, 886 and 892). It appears that the magnitude of the alteration of isotope ratios is related to the size of the shift in C/N ratios, but there are not yet enough data to permit quantification of this relationship.

All prehistoric bones have undergone diagenesis, which is the sum of the physical, chemical and biological processes that occur in the postmortem depositional environment. The results reported here do not allow identification of the types of diagenetic processes responsible for the observed shifts in bone collagen elemental and isotope ratios. Clearly, any postmortem process that adds a component to or removes a component from bone collagen will affect the collagen elemental and isotope ratios if (1) enough of the component was added or subtracted and (2) the component had a C/N ratio and $^{13}\text{C}/^{12}\text{C}$ and/or $^{15}\text{N}/^{14}\text{N}$ ratios sufficiently different from the values that characterized the collagen *in vivo*.

It is possible to be more specific with regard to one type of postmortem process that affected five of the bones listed in Table 1. These bones were burned prehistorically. Of the five, four have collagen C/N ratios outside the 2.9–3.6 range and have isotope ratios that are not indicative of the animals' feeding behaviour (882, 905, 915 and 939). The fifth bone (925), with a collagen C/N ratio of 3.3, has retained a record of the animal's feeding habits in its isotope ratios. These observations are consistent with the results of a simulation of the effects of heating on bone collagen isotope and elemental ratios. In that study¹⁹, fresh bones were heated to temperatures that would have been reached during cooking, cremation, or other types of prehistoric heating episodes (fires in midden areas, for example). Collagen extracted from the heated bones with C/N ratios in the 2.9–3.6 range had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within 1‰ of the values for collagen from unheated control bones, whereas the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for collagen from heated bones with C/N ratios outside this range were shifted by as much as 5‰.

I have adopted an operational definition of collagen in this study, defining it as that fraction solubilized by treatment with 0.001 M HCl at 90 °C for 10 h after bone powder is treated with 1.0 M HCl at room temperature for 20 min, washed to constant pH, treated with 0.125 M NaOH at room temperature for 20 h, and again washed to constant pH⁵. Clearly, the material isolated from some of the prehistoric bones, with C/N ratios outside the 2.9–3.6 range, is not collagen in the true sense but an organic material produced during postmortem heating and/or diagenesis. I refer to it as collagen until future characterization allows for accurate nomenclature.

Until further understanding of the diagenetic processes responsible for isotope shifts of the magnitude reported here is forthcoming, I suggest that the following restriction be applied to reconstructions of prehistoric diet based on bone collagen isotope ratios. Prehistoric collagen samples with C/N ratios outside the range of values observed in fresh bone collagen should be eliminated from such studies because their $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ values might have been shifted substantially and thus their use in dietary reconstruction might lead to erroneous conclusions. The application of this criterion will probably lead to the elimination of some samples with isotope ratios that have not been altered diagenetically, but it will ensure that those samples that have suffered large alterations will also be eliminated.

It is impossible to apply this criterion to most previous studies in which bone collagen isotope ratios were used to reconstruct

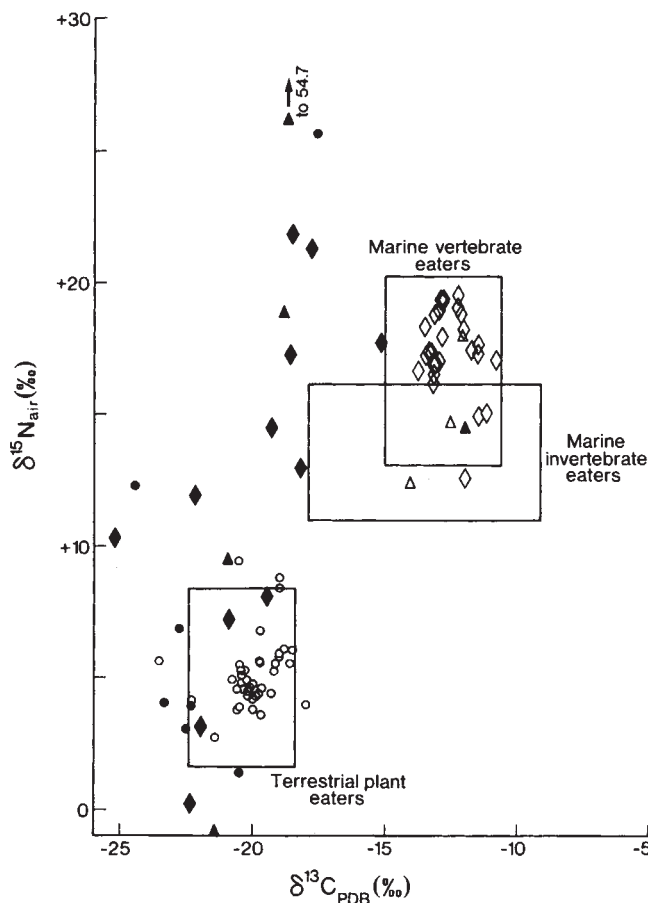


Fig. 1 Carbon and nitrogen isotope ratios of bone collagen from prehistoric terrestrial herbivorous mammals (circles) and marine carnivorous mammals (diamonds and triangles—the former indicating animals that fed predominantly on vertebrates, the latter indicating those belonging to taxonomic groups that include species feeding predominantly on invertebrates or on vertebrates). Open symbols indicate collagen samples with C/N ratios between 2.9 and 3.6, while solid symbols indicate those with C/N ratios outside this range. The boxes indicate the means $\pm$ 2 s.d. of the isotope ratios for modern mammals with the indicated habitats and feeding preferences¹⁴. In calculating the distributions for modern animals, I did not include terrestrial herbivores that probably ate substantial quantities of C_4 plants, as C_4 plants were unlikely to have been a significant food source for any of the prehistoric animals included in this study. Modern and prehistoric marine carnivores feeding in environments with substantial amounts of freshwater- and/or coral reef-based biomass are also not included in the figure, for reasons discussed in the text.

prehistoric diet because all but two of them dealt only with carbon isotope compositions and thus the C/N ratios were not measured. Of the two exceptions that involved both nitrogen and carbon isotope analyses, one¹¹ did not contain information on the collagen C/N ratios and the other⁵ contained C/N ratios that were calculated incorrectly. All the C/N ratios for prehistoric collagen samples from the former study (M. J. Schoeninger and M. J. DeN., unpublished data) and all but one from the latter, when calculated correctly²⁰, fall in the range 3.1–3.6. Thus, by the criterion proposed here, these collagens have not undergone the type of diagenetic alteration that produced the large shifts in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of some of the collagen samples analysed in this study.

This is not to say that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of prehistoric bone collagen samples with C/N ratios between 2.9 and 3.6 have not undergone diagenetic alteration. It is possible that they have, but the results presented here suggest that such diagenetic shifts must be small enough for identification of the basic feeding behaviour of the animals from their bone collagen isotope ratios to be possible. Some other approach must be devised to deter-

mine whether or not diagenetic shifts of prehistoric bone collagen isotope ratios smaller than those reported here have occurred.

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Voltage-dependent calcium and potassium channels in retinal glial cells

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Glial cells, which outnumber neurones in the central nervous system, have traditionally been considered to be electrically inexcitable and to play only a passive role in the electrical activity of the brain¹. Recent reports have demonstrated, however, that certain glial cells, when maintained in primary culture, possess voltage-dependent ion channels²⁻⁴. It remains to be demonstrated whether these channels are also present in glial cells *in vivo*. I show here that Müller cells, the principal glial cells of the vertebrate retina, can generate 'Ca²⁺ spikes' in freshly excised slices of retinal tissue. In addition, voltage-clamp studies of enzymatically dissociated Müller cells demonstrate the presence of four types of voltage-dependent ion channels: a Ca²⁺ channel, a Ca²⁺-activated K⁺ channel, a fast-inactivating (type A) K⁺ channel and an inward-rectifying K⁺ channel. Currents generated by these voltage-dependent channels may enhance the ability of Müller cells to regulate extracellular K⁺ levels in the retina and may be involved in the generation of the electroretinogram.

Recordings were made from Müller cells of the salamander *Ambystoma tigrinum* (aquatic stage) as described previously^{5,6}. Preparations were maintained at ~15 °C in perfusate containing (in mM): NaCl, 110; KCl, 2.5; CaCl₂, 2; MgCl₂, 1; HEPES, 5; dextrose, 5; equilibrated with 100% O₂ (pH 7.4). KCl was substituted for NaCl in high-[K⁺] perfusate⁶. Recordings were made from preparations within 2 h of isolation.

Müller cells have a high resting membrane conductance to K⁺ (ref. 6) which would shunt out other ionic currents, were they present. To unmask possible voltage-dependent currents, the K⁺ conductance of Müller cells in retinal slices was blocked by pressure-injecting a saturated solution of Cs⁺ and tetraethylammonium (TEA), both K⁺-channel blockers, from recording micropipettes. (External application of Ba²⁺, TEA or 4-

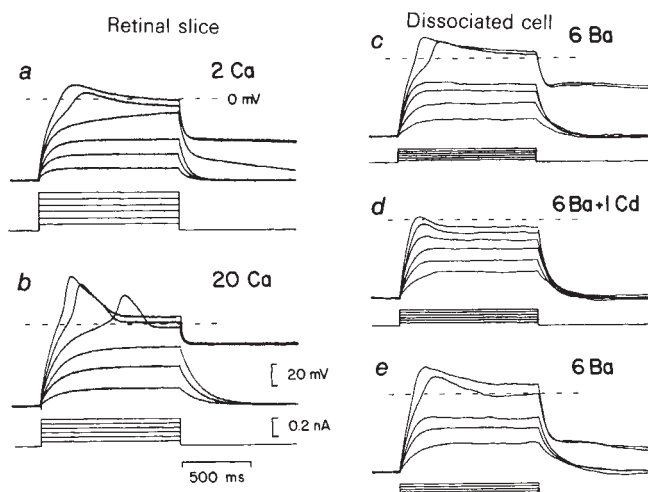


Fig. 1 Regenerative 'Ca²⁺ spikes' evoked by depolarizing current pulses in Müller cells of the salamander. *a, b*, Voltage recordings from a cell in a retinal slice. The regenerative response in control perfusate (*a*) increased significantly when [Ca²⁺] was raised from 2 to 20 mM (*b*). The plateau of the action potential lasted for several seconds following stimulation. The cell was pressure-injected with Cs⁺ and TEA to reduce the resting K⁺ conductance and bathed in pH 6.8 perfusate to de-couple the cell from other Müller cells. *c-e*, Recordings from a dissociated Müller cell in 6 mM Ba²⁺. Current pulses evoked a large sustained Ba²⁺ spike (*c*) which was substantially reduced by addition of 1 mM Cd²⁺ (*d*). The regenerative response returned when the Cd²⁺ was washed out (*e*). In both cells the resting potential was maintained at -70 mV by injection of small continuous currents. Current pulse amplitudes are indicated below each series of traces.

aminopyridine (4-AP) did not block the K⁺ conductance of Müller cells in the slice preparation.)

Müller cells in retinal slices were penetrated in their endfeet (in the nerve fibre layer) and stimulated by passing current pulses through the recording electrode. The cells, which are normally coupled by gap junctions⁷, were uncoupled by lowering the perfusate pH to 6.8 (by addition of 5% CO₂). (Application of low pH perfusate was accompanied by an increase in cell input resistance, indicating that the cells were successfully uncoupled.)

Depolarizing current pulses evoked a regenerative response in Cs⁺, TEA-injected Müller cells bathed in pH 6.8 perfusate (Fig. 1*a*). This response was significantly increased when perfusate [Ca²⁺] was raised from 2 to 20 mM (*n* = 10; Fig. 1*b*). The regenerative response was caused by an influx of Ca²⁺ triggered by cell depolarization: the Ca²⁺ spikes were not diminished by addition of 1 μM tetrodotoxin (TTX), a Na⁺-channel blocker, but were reduced more than 70% by 60 μM verapamil, a selective Ca²⁺-channel blocker⁸.

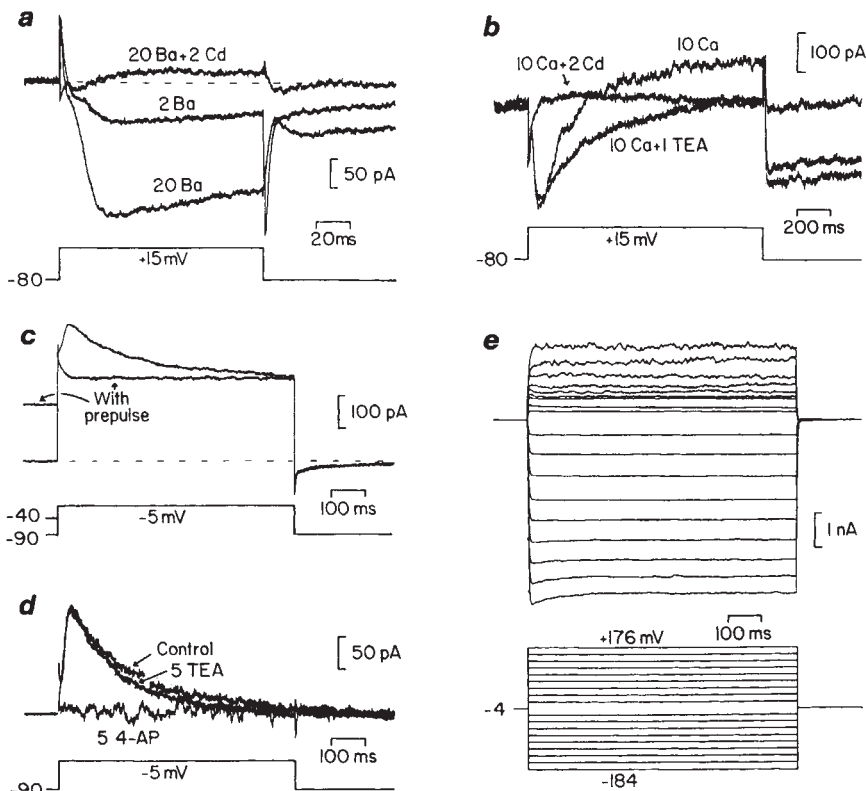
To investigate this voltage-dependent current in more detail, additional recordings were made from enzymatically dissociated Müller cells of salamanders⁶. Recordings were made with patch-clamp electrodes (filled with 125 mM KCl) used in the 'whole cell' recording mode⁹.

When dissociated Müller cells were bathed in 6 mM Ba²⁺, which effectively blocked the resting K⁺ conductance, large regenerative spikes were evoked by depolarizing current pulses (*n* = 25; Fig. 1*c*). This response was substantially reduced by addition of 1 mM Cd²⁺, a potent Ca²⁺-channel blocker¹⁰ (Fig. 1*d*) and recovered following removal of the Cd²⁺ (Fig. 1*e*). The regenerative response was also blocked by 50 μM verapamil, but was unaffected by 1 μM TTX.

The voltage-dependent currents present in Müller cells were further characterized with voltage-clamp recordings from dissociated cells. Single electrode clamps were made with patch electrodes in the 'whole cell' recording configuration⁹.

When cells were bathed in Ba²⁺ and TEA (both K⁺ channel blockers), a long lasting voltage-dependent inward current was recorded (*n* = 21). This current increased significantly when

Fig. 2 Voltage-clamp records of voltage-dependent currents in dissociated salamander Müller cells. **a**, Inward Ca^{2+} current. The cell potential was stepped to the value which evoked maximal inward current (+15 mV in 20 mM Ba^{2+} and +5 mV in 2 mM Ba^{2+}). The inward current increased when $[\text{Ba}^{2+}]$ was raised from 2 to 20 mM and was abolished by addition of 2 mM Cd^{2+} . The perfusate contained 5 mM TEA to reduce Ca^{2+} -activated K^{+} currents. Recordings made from a cell with endfoot intact. **b**, Outward Ca^{2+} -activated K^{+} current. In 10 mM Ca^{2+} perfusate (10 Ca), a slowly developing outward current was superimposed on the inward Ca^{2+} current. 1 mM TEA reduced the outward current while 2 mM Cd^{2+} eliminated both the inward and outward currents. Recordings from a cell lacking its endfoot process. **c**, Transient outward (type A) K^{+} current. The transient current was eliminated when the voltage step was preceded by a 2-s prepulse to -40 mV. **d**, Transient current shown in isolation by subtracting records preceded by a prepulse from records without a prepulse. The 'control' is the difference of the two records in **c**. The transient current in control perfusate was unaltered by 5 mM TEA but abolished by 5 mM 4-AP. Records from **c** and **d** were from the same endfoot-shorn Müller cell. **e**, Inward-rectifying K^{+} current evoked by depolarizing and hyperpolarizing voltage steps in 80 mM K^{+} perfusate. Holding potential (E_h), -4 mV, the membrane potential of the cell in 80 mM K^{+} . Recordings from an endfoot-shorn cell. In **a-e**, inward currents are shown as downward deflections. Capacitive transients were reduced using transient cancellation circuitry. In **a** and **b**, linear contributions of the 'leakage current' were removed by subtracting a scaled version of records evoked by depolarizations to ~ -60 mV.



external Ba^{2+} (which passes freely through Ca^{2+} channels^{10,11}) was raised from 2 to 20 mM (Fig. 2a). The inward current was blocked by 2 mM Cd^{2+} (Fig. 2a) and 50 μM verapamil. The current-voltage (I - V) relation of the inward current, recorded in 2 mM Ba^{2+} (Fig. 3a, Ca) peaked at +5 mV and had a (chord) conductance of 2.6 ± 1.7 nS (mean $\pm$ s.d.) at 0 mV ($n=3$). These characteristics are typical of current generated by voltage-dependent Ca^{2+} channels^{10,11}.

In perfusate containing either 2 or 10 mM Ca^{2+} , depolarizing voltage steps to $\sim +15$ mV evoked a transient inward current followed by a slowly developing outward current ($n=14$; Fig. 2b, '10 Ca'). The outward current was partially blocked by 1 mM TEA (known to block Ca^{2+} -activated K^{+} channels) while 2 mM Cd^{2+} blocked both the inward Ca^{2+} current and the outward current (Fig. 2b). The I - V relation of the sustained outward current (Fig. 3a, K_{Ca}) had a prominent N-shape, characteristic of current generated by Ca^{2+} -activated K^{+} channels¹². Conductance was 2.7 ± 1.0 nS at 0 mV and 6.3 ± 3.2 nS at +50 mV ($n=3$).

A second outward current, which peaked at ~ 40 ms and decayed within 1 s, was evoked by depolarizing voltage steps to ~ -5 mV ($n=5$). This outward current could be isolated from inward Ca^{2+} current by recording from cells for more than 20 min, at which time the Ca^{2+} current was diminished. The transient outward current was completely inactivated by a 2-s prepulse to -40 mV (Fig. 2c). It was abolished by 5 mM 4-AP (known to block fast-inactivating K^{+} channels) but was unaffected by 5 mM TEA (Fig. 2d). The I - V relation of the transient current (Fig. 3a, K_A) shows that it turns on at between -60 and -40 mV. Conductance was 1.8 ± 0.7 nS at 0 mV ($n=5$). These properties demonstrate that the current is generated by fast-inactivating (type A) K^{+} channels^{13,14}.

A third type of K^{+} current was recorded from Müller cells when the K^{+} concentration of the perfusate was raised above control level ($n=6$). A series of voltage-clamp records in 80 mM K^{+} (Fig. 2e) shows that evoked current was much larger when the cell was hyperpolarized than when it was depolarized. This inward rectification is shown clearly in the I - V relation of a

cell (Fig. 3b) which was bathed sequentially in perfusate containing 2.5, 16 and 80 mM K^{+} . The I - V curves are typical of current arising from inward-rectifying K^{+} channels¹⁵. Conductance was 7.1 ± 2.4 nS in 2.5 mM K^{+} at -120 mV ($n=4$) and 31 ± 5.6 nS in 80 mM K^{+} at -100 mV ($n=3$).

The magnitudes of the four voltage-dependent currents were approximately the same whether they were recorded from cells having intact endfeet or from cells whose endfeet had been shorn off during the dissociation procedure. Thus, the channels producing these four currents were not localized principally in the endfoot process of the cell. Except where indicated, all recordings reported here were made from cells shorn of their endfeet in order to eliminate the large, voltage-independent K^{+} conductance which is localized in the endfoot process^{5,6}. (The conductance of Müller cells at resting potential is 127 nS in intact cells and 6.6 nS in cells shorn of their endfeet⁶.)

The findings described here demonstrate that four types of voltage-dependent ion channels are present in freshly dissociated Müller cells of salamanders: Ca^{2+} channels, Ca^{2+} -activated K^{+} channels, type A K^{+} channels and inward-rectifying K^{+} channels. At least one of these, the Ca^{2+} channel, is also present in Müller cells in retinal slices and thus probably occurs *in vivo* as well. The results confirm findings from cultured astrocytes, where Ca^{2+} channels³ and Ca^{2+} -activated K^{+} channels¹⁶ have recently been reported. Inward rectification has also been noted in turtle Müller cells⁷. Axonal-type Na^{+} channels and delayed rectifier K^{+} channels, reported in primary cultures of Schwann cells² and astrocytes^{4,17}, were not observed in the present study.

Of the four voltage-dependent ion channels found in Müller cells, the inward-rectifying K^{+} channel may be the most important functionally, as it is the only one that appears to be open in normal physiological conditions. This channel may be involved in regulating extracellular K^{+} levels, $[\text{K}^{+}]_o$, in the retina: K^{+} is thought to flow into Müller cells in regions of light-evoked $[\text{K}^{+}]_o$ increase and to flow out through the Müller cell endfeet, which contain 95% of the total cell membrane conductance⁶. This process, which has been termed K^{+} siphoning¹⁸, functions to transfer excess K^{+} directly from the retina to the vitreous

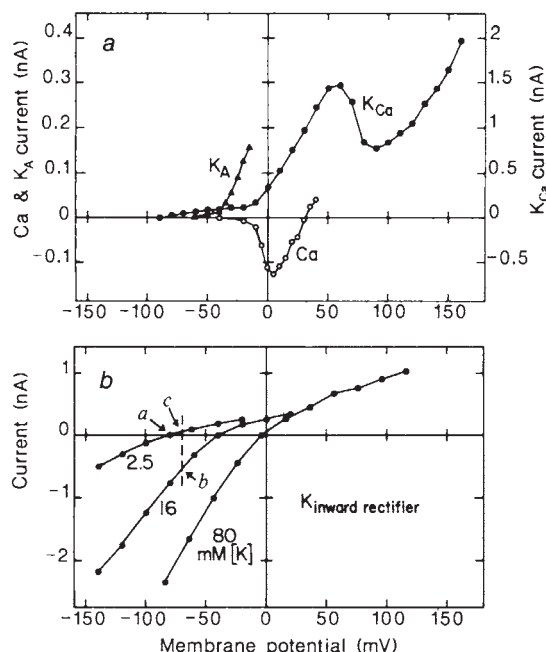


Fig. 3 Current-voltage plots of the voltage-dependent ionic currents illustrated in Fig. 2. *a*, The Ca^{2+} current relation (Ca) was obtained from an intact Müller cell bathed in perfusate containing 2 mM Ba^{2+} and 5 mM TEA. It represents peak inward current, corrected for an extrapolated linear leakage current. E_h , -80 mV. The Ca^{2+} -activated K^+ current relation (K_{Ca}) was obtained from an endfoot-shorn cell in control perfusate and represents the steady-state current attained during a 3.5-s voltage pulse. The small outward current on the K_{Ca} curve between -80 and -30 mV is inward rectifier current. E_h , -90 mV. The transient (type A) K^+ current relation (K_A) was obtained from an endfoot-shorn Müller cell in control perfusate. Current amplitude was measured between the peak of the response and the value at the end of an 800 ms pulse. E_h , -90 mV. *b*, The inward rectifier K^+ current relation was recorded from an endfoot-shorn cell bathed sequentially in 2.5, 16 and 80 mM K^+ perfusate. Current amplitudes represent the maximal values attained during an 800-ms voltage step. E_h , -80, -40 and -4 mV, the cell membrane potential in the three perfusates. The dashed line and points *a*-*c* in *b* are used to illustrate changes in membrane conductance caused by a localized $[K^+]_o$ increase. See text for details. Note differences in the vertical scales for the four currents.

humor. The effectiveness of K^+ siphoning is limited by the low resting conductance of the cell body membrane of Müller cells ($137 \mu S cm^{-2}$, ref. 6). However, the voltage and K^+ -dependence of the inward-rectifying K^+ channel may lead to an increase in this membrane conductance when $[K^+]_o$ rises.

The effect of inward-rectifying K^+ channels on K^+ siphoning by Müller cells can be appreciated by considering the I - V relation of the channel. If, for example, $[K^+]_o$ is raised from 2.5 to 16 mM in a localized region of the cell, the I - V relation of those channels exposed to the K^+ increase will be shifted to the right (Fig. 3*b*, curves 2.5 and 16). The membrane potential of the cell (dashed vertical line in Fig. 3*b*) will be depolarized by the $[K^+]_o$ increase, but not to the degree predicted by the new K^+ equilibrium potential; the high conductance of the Müller cell endfoot has the effect of clamping the membrane potential at near the resting level (see ref. 6). The conductance of the inward-rectifying channels exposed to 16 mM K^+ (given by the slope of the I - V curve at the point where it intersects the vertical line, Fig. 3*b*, point *b*) is larger than at rest (Fig. 3*b*, point *a*). In this example, the conductance is 4.2 times the resting value. At the same time, the I - V relation of those channels not exposed to the $[K^+]_o$ increase remains unshifted (Fig. 3*b*, curve 2.5). Because the cell is partially depolarized by the $[K^+]_o$ increase, however, the conductance of these channels will be smaller (by 20%) than at rest (as indicated by the slope of the I - V curve at point *c* compared with the slope at point *a*, Fig. 3*b*).

Note that, in physiological conditions, the changes in inward rectifier conductance will probably be less than those illustrated here, as light-evoked increases in retinal $[K^+]_o$ are not thought to reach 16 mM (ref. 19).

Thus, the properties of inward-rectifying K^+ channels enhance the process of K^+ siphoning by increasing membrane conductance in regions of $[K^+]_o$ increase, leading to a greater influx of K^+ into Müller cells, and by reducing membrane conductance in other regions of the cell, reducing the amount of K^+ deposited in adjacent retinal tissue. (Efflux of K^+ from the endfoot process will not be reduced by cell depolarization as it occurs through K^+ channels which are largely voltage-independent).

The functions of the other three types of voltage-dependent channels reported here are less clear, as they normally remain closed. However, large increases in $[K^+]_o$, such as encountered during spreading depression episodes²⁰, might depolarize Müller cells sufficiently to open these channels. The resulting increase in membrane K^+ conductance would aid in clearing K^+ from the retina by augmenting siphoning currents.

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A single stem cell can recolonize an embryonic thymus, producing phenotypically distinct T-cell populations

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There is much interest in early T-cell development, particularly in relation to the diversification of the T-cell receptor repertoire^{1,2} and the elucidation of the lineage relationships between T-cell populations in the thymus and peripheral lymphoid organs (reviewed in refs 3, 4). However, the requirements for the growth of the earliest thymic T-cell precursor in 13-14-day mouse embryo thymus in isolation from the thymic environment are unknown. Proliferation and maturation of such cells are not sustained either in the presence of monolayers of thymic stromal cells (refs. 5, 6 and see below) or by the addition of interleukin-2 (IL-2), despite the expression of receptors for this growth factor on a proportion of thymocytes displaying the immature $Thy 1^+ Lyt-2^- L3T4^-$ phenotype in the embryonic thymus^{7,8}. In contrast, when maintained within the intact thymic environment in organ cultures^{9,10}, 13-14-day thymic stem cells do show a pattern of surface marker and functional development similar to that seen *in vivo*, suggesting that short-range growth signals, perhaps necessitating direct contact with organized epithelial cells, are required. We have shown, by exploiting the selective toxicity of deoxyguanosine

(dGuo) for early T cells, that this organ culture system can be manipulated to produce alymphoid lobes that can be recolonized from a source of precursors in a transfilter system¹¹. We now show that recolonization of alymphoid lobes can also be achieved by association with T-cell precursors in hanging drops, allowing recolonization by exposure to defined numbers of precursors, including a single micromanipulated stem cell. Analysis of T-cell marker expression in these cultures shows that a single thymic stem cell can produce progeny of distinct phenotypes, suggesting that these marker-defined populations are not derived from separate pre-thymic precursors, but arise within the thymus.

The embryonic thymus rudiment at day 14 of gestation provides a convenient source of immature T-cell precursors for thymus recolonization. Teasing these rudiments apart produces suspensions of large blast-like cells, many of which have begun to express Thy-1 but lack the Lyt-1, Lyt-2 and L3T4 markers of later stages^{9,12}. When these cells are placed in hanging drops in the presence of alymphoid thymic lobes, but are prevented from making contact with them (Fig. 1a), they fail to develop, reinforcing the view that close-range interaction with the thymic environment may be needed in addition to any requirement for diffusible growth factors. If, however, they are allowed to come into proximity with the lobes (Fig. 1b), recolonization occurs in some cases, perhaps through chemotactic attraction of the precursors by factors emanating from the thymic epithelium, as suggested for the recolonization of the thymic anlage *in vivo*¹³. Once within the thymus, proliferation and maturation of the colonizing cells, as defined by the appearance of T-cell markers, occurs to produce progeny that can be shown to be exclusively derived from the donor precursors using strain combinations differing at the *Thy-1* locus (data not shown). Using this approach, we have observed that the number of lobes recolonized is related to the number of precursors in the hanging drop and decreases from ~80% with 2,000 precursors per well to 10% with 50–75 precursors per well. This presumably reflects the reduced likelihood of effective collisions between the lobe and cells of thymus-colonizing potential, although it is not clear whether this is entirely due to the relative disposition of the lobe and the precursors within the hanging drop or whether it also reflects heterogeneity of 14-day blast cells in terms of their thymus-colonizing potential.

Despite the decreasing success rate for recolonization as the number of precursors is reduced, these observations raised the possibility of achieving recolonization using a single precursor. To investigate this, individual blast cells from fresh 14-day thymus lobes were selected by micropipette and placed in hanging drops with an alymphoid dGuo-treated thymic lobe. Association in this manner resulted in successful recolonization in 2–3% of cases in experiments involving over 300 lobes. Development of a single precursor over a total culture period of 12 days resulted in minimum estimates of progeny numbers of 2×10^4 , implying an average doubling time of 17–21 h (Tables 1, 2). These cells were identified as derivatives of the single donor by the presence of donor Lyt antigens (Table 1). Table 1 also shows that a single precursor can give rise to progeny with three different Lyt phenotypes, a major Lyt 1⁺2⁺ population and smaller populations of Lyt 1⁺2⁻ and Lyt 1⁻2⁺ cells. Although we could distinguish all these phenotypes in the fluorescence microscope, flow cytometry studies³ have indicated that some Lyt-1, unlike Lyt-2, is present on all T cells, reducing the value of this marker in identifying T-cell subpopulations. Our subsequent experiments have therefore used the L3T4 marker; this is present in conjunction with Lyt-2 on most thymocytes but is exclusive to helper-type cells in mature populations¹².

Dual labelling with L3T4 and Lyt-2 revealed that in some lobes three marker phenotypes—L3T4⁺ Lyt-2⁺; L3T4⁺ Lyt-2⁻; and L3T4⁻ Lyt-2⁻—were generated from a single cell (Table 2, Fig. 2). In other lobes the L3T4⁺ Lyt-2⁻ populations were not detected, although the other populations were present. The reason for the absence of this population from some lobes and for the variation in size of the different populations between lobes is unclear, although variability in the populations defined

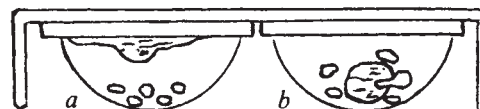


Fig. 1 Association of T-cell precursors with alymphoid thymic lobes in hanging drops. Alymphoid thymic lobes were prepared by organ culturing embryonic thymus lobes isolated on day 14 of gestation in medium containing 1.35 mM dGuo as described previously¹¹. This eliminates developing T cells while leaving the major epithelial components intact²³. Donor thymic stem cells were obtained by teasing apart fresh 14-day lobes with fine cataract knives in a pool of medium. The resultant suspension was counted and adjusted to give the required cell numbers in 10 μ l. Hanging drop cultures were prepared in Terasaki plates by adding 10 μ l of medium (Dulbecco's modified Eagle's medium + 10% fetal calf serum) to each well together with an alymphoid lobe. In some instances (a) the plates were incubated overnight in an upright position to allow attachment of the alymphoid lobe to the floor of the well. Wells containing either a lobe attached to the floor or a freshly added lobe then received a further 10 μ l of medium containing the precursors and the plates were immediately inverted to form hanging drops. This resulted either in separation of the lobe and the precursors at opposite poles of the drop (a) or in their approximation at the tip of the drop (b). In some experiments, before inversion of the plate, 20- μ l drops containing a freshly added alymphoid lobe received a single precursor selected and transferred into the drop using a finely drawn mouth-controlled pipette. Observations of wells containing separated lobes and precursors over a 5–12-day culture period showed no evidence of lymphoid cell development and did not produce recoverable yields, although the stromal cells of the lobe remained viable and spread over the plastic 'ceiling' of the drop. Where precursors and lobe were allowed to make contact (b), colonization of the lobe occurred in a proportion of cases, resulting in lymphocyte proliferation and maturation as defined by cell counts and the sequential appearance of the T-cell markers Thy-1, Lyt-1 and Lyt-2. Although preliminary experiments showed that this development could proceed in hanging drops over a 10–12-day culture period, it was less satisfactory than that obtained in organ culture. Consequently, in all experiments reported here lobes were removed from the hanging drops after 2 days, washed and placed in organ culture for a further 10 days to allow development to proceed in optimal *in vitro* conditions.

Table 1 Cell yields and Lyt marker expression on cell populations obtained from alymphoid CBA (Lyt 1.1 Lyt 2.1) thymic lobes recolonized by a single BALB/c (Lyt 1.2 Lyt 2.2) thymic stem cell

Cell count $\times 10^4$	% Lyt 1 ⁺ 2 ⁺	% Lyt 1 ⁺ 2 ⁻	% Lyt 1 ⁻ 2 ⁺	Null
4.8	54.2	19.8	8.3	17.7
10.9	65.4	18.7	7.5	8.4
11.7	57.9	7.4	15.9	18.8
7.2	42.7	15.2	13.5	28.6

Lobes recolonized by a single stem cell were produced as described for Fig. 1. After 12 days of culture, suspensions were produced by mechanical dispersion of the lobes and counted and dual immunofluorescence staining carried out to identify Lyt-expressing populations of donor origin. First-step reagents were anti-Lyt-1.2 (mouse IgG2b; NEN) and anti-Lyt-2.2 (mouse IgM; NEN); second-step reagents, anti-mouse IgG2-F1 and anti-mouse IgM (Fc)-Rh (Nordic). The results indicate the expression of Lyt antigens of donor type, demonstrating successful colonization and differentiation by a single precursor.

by these markers is also seen in non-manipulated organ cultures¹⁰. In the case of single-cell-recolonized lobes, one possibility is that some colonizing cells have more limited potential than others. Alternatively, variation in culture conditions between individual lobes may have an effect. However, the fact that all three populations were observed in some lobes demonstrates that there are cells present in the 14-day fetal thymus which are able to give rise to all three phenotypes; this argues against determination of the various marker-defined thymocyte lineages at a pre-thymic level. The crucial next step will be to determine the functional phenotypes generated from a single cell. Pre-

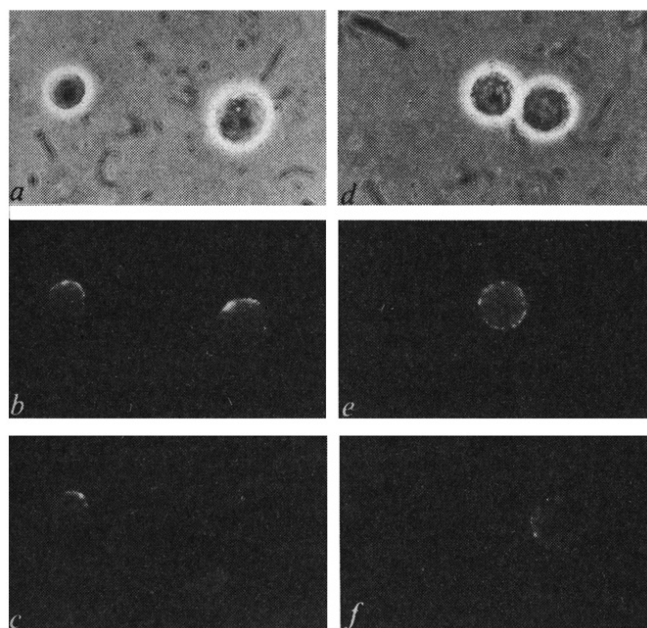


Fig. 2 L3T4 and Lyt-2 antigen expression on cells derived from a single-cell-recolonized thymus lobe demonstrates that a single thymic stem cell can give rise to three marker-defined phenotypes. Lobes recolonized by a single thymic stem cell were produced as described in Fig. 1. Dual immunofluorescence labelling was carried out using the reagents described for rows 5–7 in Table 2. *a–c*, Two cells are shown (*a*), the smaller of which labels with both Lyt-2 (*b*) and L3T4 (*c*), whereas the larger cell expresses only Lyt-2 (*b*). *d*, Two adjacent lymphoid cells from the same preparation as that in *a–c*. These cells express Lyt-2 (*e*) and L3T4 (*f*) in a mutually exclusive fashion. Thus, all three phenotypes—L3T4⁺ Lyt-2⁺; L3T4⁺ Lyt-2[−]; and L3T4[−] Lyt-2⁺—were present in this lobe.

Table 2 Lyt2 and L3T4 marker expression on the progeny of a single thymic stem cell

Cell count ×10 ⁴	%Lyt 2.2 ⁺ L3T4 ⁺	%Lyt 2.2 ⁺ L3T4 [−]	%Lyt 2.2 [−] L3T4 ⁺	Null
4.2	22.1	9.7	0	68.2
2.0	40.5	17.1	4.5	37.9
6.6	50.5	25.2	0	24.6
1.95	15.1	44.2	1.2	31.5
5.0	37.5	19.2	0	43.3
3.0	20.8	15.8	0	63.6
8.8	52.7	14.5	4.4	28.4

Strain combinations and details for rows 1–4 are as in Table 1. Immunofluorescence reagents for this group were: first step, anti-Lyt 2.2 (mouse IgM) and anti-L3T4 (clone H129.19 rat IgG²²); second step, anti-mouse IgM(Fc)-Rh and anti-rat IgG(Fc)-F1 (Nordic). In rows 5–7 the strain combination consisted of AKR stem cell donor and BALB/c host thymus, and the following reagents were used sequentially: anti-L3T4 (H129.19) anti-rat IgG(Fc)-Rh, 1% normal rat serum wash, anti-Lyt-2-ars (clone 53-6.7), anti-ars-FL. All reagents were tested for specificity and non-cross-reactivity and gave the expected staining patterns on adult T cells. The results show that in both strain combinations tested, in some lobes all three phenotypes were generated, similar results being obtained with the two different sets of reagents used.

liminary studies have shown that the progeny of a single cell can respond to concanavalin A without added growth factors, implying that they can produce their own IL-2 and are capable of helper-type activity. If the same cultures can also generate cells possessing cytotoxic activity, this will indicate that both functional phenotypes can arise from a single cell irrespective of their relationship to the marker-defined populations.

Previous studies on thymocyte populations in bone-marrow-reconstituted, irradiated adults have suggested that entire thymus lobes can be recolonized by the progeny of a small number of donor bone marrow cells^{14,15}. However, the initial pattern of

differentiation of donor-derived cells in this model seems to differ from that seen in ontogeny^{16,17}, and it is not clear whether all the recolonizing cells are equivalent to those populating the thymus during normal development. Our demonstration that a single immature fetal thymus cell can repopulate a thymus lobe, coupled with observations suggesting limited entry of precursors into the thymus during embryonic¹⁸ and adult life¹⁹, is consistent with the need for diversification of the T-cell receptor repertoire within the thymus. Consistent with this, recent studies have shown that thymus development is associated with an increasing proportion of cells showing rearrangement at the β -chain locus¹. However, the possibility of some receptor gene rearrangement at a pre-thymic level^{20,21} and the progressive recruitment of rearranged cells into the developing thymus cannot be excluded. In this context, examination of the pattern of gene rearrangement in the progeny of a single intra-thymic stem cell should show whether there is extensive diversification within the thymus or whether, despite considerable proliferation, only limited rearrangements are generated in the descendants of a single precursor.

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Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase-negative phenotype

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Recent advances in molecular genetics have led to the possibility of using large DNA viruses, such as vaccinia virus, as a biological delivery system for immunizing man against unrelated disease-causing agents^{1–7}. When live vaccinia virus recombinants expressing the hepatitis B virus surface antigen (HBsAg)^{8,9}, the influenza A virus haemagglutinin¹⁰, the herpes simplex virus (HSV) type 1 D glycoprotein^{11,12}, the rabies virus G glycoprotein^{13,14} and the vesicular stomatitis virus G glycoprotein¹⁵ were used for immunization, animals were protected upon challenge with the appropriate

pathogenic agent. A major concern with using such vaccines, however, stems from the previously documented vaccinia virus-associated post-immunizing complications¹⁶. We present here experimental evidence that thymidine kinase-negative (TK⁻) vaccinia virus recombinants, constructed by inserting a variety of DNA coding sequences into the vaccinia virus *tk* gene, are less pathogenic for mice than wild-type virus.

The vaccinia virus *tk* locus^{4,17} was chosen as a site of insertion for foreign DNA, primarily because it provides an efficient method of selecting infectious recombinants^{6,17}. However, the possibility that this would lead to attenuation of virus virulence was considered because of data obtained with herpesviruses. HSV types 1 and 2 and marmoset herpesvirus encode a TK that is important for virus pathogenicity in certain strains of inbred mice inoculated by the intracerebral or intradermal routes^{18,19}. This effect has been attributed to the role of TK in enhancing herpesvirus replication in non-dividing cells²⁰⁻²². To determine whether inactivation of the vaccinia virus *tk* gene by the insertion of foreign DNA led to attenuation, several independently derived TK⁻ recombinant viruses produced from two closely related TK⁺ strains of vaccinia virus (WR and Wyeth) were inoculated into BALB/cByJ mice by the intracerebral (i.c.), intraperitoneal (i.p.) and intradermal (i.d.) routes. Wyeth is the New York City Board of Health (NYCBH) strain used extensively in the United States and elsewhere for smallpox vaccination. WR, derived from the NYCBH strain by multiple passage in mouse brain²³, has been more extensively studied in the laboratory, and because of its enhanced virulence provides a more sensitive model for studying virus attenuation.

Table 1 lists the amount of virus required to kill half of the mice (50% lethal dose, LD₅₀) by the i.c. route of inoculation. The WR strain TK⁻ vaccinia virus recombinants, which encoded the genes for the haemagglutinin of influenza virus A/Japan/305/57 (vInf1)¹⁰ and herpes simplex virus (strain KOS) type I D glycoprotein (v52)¹² had an LD₅₀ 2.5 × 10⁴–10⁵-fold higher than that of wild-type (WT) virus. Mice that survived i.c. inoculation of WR-WT, WR-vInf1 or WR-6/2 viruses showed no signs of delayed onset of disease by 24 days post-infection, at which time the experiment was terminated. Significantly, insertion of DNA into the *tk* locus of the Wyeth strain of vaccinia virus also had an attenuating effect. The LD₅₀ of Wyeth-v55 (expressing HBsAg) was even higher than that of the WR recombinants because of lower starting virulence of this vaccine strain. Since TK11 (a gift of R. Condit), which lacks a functional *tk* gene but contains no insertion of foreign DNA, showed a higher LD₅₀ than WT, the TK⁻ phenotype and not the foreign gene expression must be the major factor contributing to lowered virus virulence. In summary, insertion of foreign DNA into the *tk* locus attenuates strains of vaccinia virus that vary widely in neurovirulence.

It is of interest that the mutant designated 6/2 (ref. 24), which has a spontaneous 9-kilobase deletion located proximal to the left inverted terminal repetition, yielded the highest LD₅₀ by the i.c. route of any WR strain derivatives examined. A previous study showed that the region of the WT virus genome defined by the 6/2 deletion is also suitable for the insertion and expression of foreign DNA⁶.

Decreased virus pathogenicity also was demonstrated by the i.p. route of inoculation. Lethality by this route is probably a consequence of virus seeding and replication in the internal organs. The LD₅₀ in BALB/cByJ male mice for strain WR-WT by the i.p. route was 1 × 10⁸ ± 1.1 plaque-forming units (PFU); however, doses of viruses vInf1, v52 and TK11 as high as 10⁹ PFU were insufficient to produce a lethal effect (that is, LD₅₀ > 10⁹ PFU; Table 2). A similar result was seen in C3H/HeJ mice with vInf1 virus (data not shown). In a second experiment, i.p. injection of BALB/c mice with 10⁸ PFU of WR-WT or WR-v52 resulted in 20/25 and 0/25 deaths, respectively. Surviving mice recovered rapidly from the virus infection, and as late as 49 days post-inoculation no new signs of disease were observed.

To quantitate further the differences in virulence between WR

Table 1 LD₅₀ of vaccinia and derivative viruses in BALB/cByJ male mice by i.c. inoculation

Virus strain	PFU inoculum at LD ₅₀
WR-wild type	1 × 10 ⁸ ± 1.0
WR-vInf1	4 × 10 ⁶ ± 1.4*
WR-v52	2.5 × 10 ⁵ ± 2.8*
WR-TK11	4.0 × 10 ⁴ ± 2.5*
WR-6/2	6.3 × 10 ⁶ ± 1.2*
Wyeth-wild type	3.2 × 10 ⁶ ± 1.4
Wyeth-v55	9.1 × 10 ⁷ ± 1.1*

Serial 10-fold dilutions of virus (30 µl) purified from cells³⁰ in 1 mM Tris-HCl (pH 9.0) were injected with a tuberculin syringe and 26-gauge needle into the right cerebral hemisphere of six anaesthetized 6-week-old BALB/cByJ male mice. The Spearman-Kärber method was used to calculate the average lethal dose for 50% of the population (LD₅₀)³¹. Only mice dying between the 2nd and 14th day after inoculation were used in the calculations.

* Significantly different from animals inoculated with wild-type virus; P < 0.02, Student's *t*-test.

Table 2 LD₅₀ of vaccinia wild-type and derivative viruses in BALB/cByJ male mice by i.p. inoculation

Virus strain	Virus dose*	Mortality	PFU inoculum at LD ₅₀
WR-wild type†	10 ⁹	9/10	1 × 10 ⁸ ± 1.1
	10 ⁸	7/12	
	10 ⁷	0/12	
WR-vInf1	10 ⁹	0/6	> 1 × 10 ⁹
	10 ⁸	0/6	
	10 ⁷	0/6	
WR-TK11	10 ⁹	0/3	> 1 × 10 ⁹
	10 ⁸	0/6	
	10 ⁷	0/5	

* Serial 10-fold dilutions of virus (100 µl) were injected i.p. into 6-week-old BALB/cByJ male mice. The LD₅₀ was calculated as described in Table 1 legend.

† Results of two experiments.

and Wyeth-WT viruses and TK⁻ recombinants, a direct comparison of virus infectivity in spleen and liver was carried out. The level of TK⁻ recombinant virus infectivity in spleen and liver as compared with that of WT virus at various times following i.p. inoculation is depicted in Table 3. In three independent experiments, TK⁻ recombinant vInf1, v52 and v55 viruses yielded lower levels of infectivity in spleen and liver tissue than the corresponding WT virus. This difference in infectivity levels may be best attributed to a decreased spread of recombinant virus and/or to lower rates of replication in target organs. It is of interest that the levels of infectious WR viruses in the spleen and liver were higher than that of the corresponding Wyeth viruses at all time points examined.

Further evidence supporting either decreased dissemination and/or replication of TK⁻ recombinant viruses comes from measuring the levels of vaccinia virus neutralizing antibody. At 21 days after i.p. inoculation of three groups of mice with 1 × 10⁷ PFU of WR-WT, WR-vInf1 and WR-v52, the latter two groups (which received the recombinant viruses) had significantly lower neutralizing titre than the WT group (data not shown). Taken together, the differences between TK⁻ recombinant and WT viruses in LD₅₀ values, virus infectivity in spleen and liver, and neutralizing antibody titres indicate that the TK⁻ recombinant viruses are less virulent than WT by the i.p. as well as the i.c. route of inoculation.

If the vaccinia virus recombinants are to be used as vaccines, it is important that decreased virulence does not occur at the expense of a marked drop in immunogenicity. Since vaccinia virus vaccines are administered by i.d. scarification, it was necessary to evaluate the immunogenicity and virulence of TK⁻

Table 3 Vaccinia wild-type and TK⁻ recombinant virus infectivity in liver and spleen tissue of BALB/cByJ mice by i.p. inoculation

Virus	Expt	log ₁₀ PFU per g tissue on indicated day post-inoculation*							
		1	2	3	6	1	2	3	6
WR-wild type	1	7.57 ± 0.1	9.52 ± 0.1	7.11 ± 0.1	9.41 ± 0.1	7.15 ± 0.2	8.91 ± 0.2	6.30 ± 0.4	7.18 ± 0.6
WR-vInfl	1	5.00†	5.46†	4.26 ± 0.1	6.08 ± 0.2	3.69 ± 1.5	4.11 ± 0.9	ND‡	ND
WR-wild type	2	6.77 ± 0.2	7.65 ± 0.1	5.52 ± 0.3	5.23 ± 0.8	5.30 ± 1.5	5.50 ± 0.20	ND	ND
WR-v52	2	3.53 ± 0.7	5.04 ± 0.4	3.98 ± 0.2	3.95 ± 0.1	ND	3.45 ± 0.1	ND	ND
Wyeth-wild type	3	4.08 ± 0.0	6.30 ± 0.08	ND	4.20 ± 0.01	ND	ND	ND	ND
Wyeth-v55	3	ND	5.04 ± 0.18	ND	ND	ND	ND	ND	ND

* Eight 4–6-week-old male BALB/cByJ mice were inoculated intraperitoneally with the indicated virus at a multiplicity of infection of 5×10^8 in expts 1, and 5×10^7 in expts 2 and 3. At the indicated times post-inoculation, two animals from each group were killed, and virus infectivity present in spleen and liver was quantitated by standard procedures. ND, No virus detected; detection limit log₁₀ 2.85 and 3.00 in liver and spleen, respectively.

† Based on one mouse only.

Table 4 Vaccinia virus neutralizing antibody titres in BALB/cByJ male mice inoculated i.d. with vaccinia wild-type and TK⁻ recombinant viruses

Virus strain	Reciprocal of neutralizing antibody titre at different times post-inoculation*	
	4 weeks	7 weeks
WR-wild type	4,444 ± 1.2 (1,960–10,780)	12,591 ± 1.4 (4,128–20,740)
WR-v52	5,092 ± 1.1 (2,308–8,780)	—†
WR-vHBs4	3,283 ± 1.4 (1,392–8,052)	8,523 ± 1.3 (2,528–41,792)
Wyeth-wild type	2,729 ± 1.5 (812–21,840)	2,957 ± 1.3 (2,148–10,152)
Wyeth-v55	7,834 ± 1.4 (2,215–20,960)	5,577 ± 1.5 (804–11,932)
None	<160	—†

* A group of 6–8 8-week-old male BALB/cByJ mice was inoculated with 10⁷ PFU of each of the indicated strains by scarification using 18 strokes of a 25-gauge needle through 10 µl of virus suspension placed on the dorsal surface of the base of the tail³². At 4 and 7 weeks post-inoculation, each mouse was bled from the orbital sinus. Plasma was heat-inactivated at 56 °C for 30 min, and virus neutralization assays were carried out by standard methods³³. The highest dilution of plasma at which a 50% reduction in the number of indicator virus plaques (intracellular band-purified WT virus) was chosen as the assay end-point. The geometric mean and relative standard error was calculated from six to eight neutralizing titres obtained for each inoculation series. Values in parentheses indicate the titre range.

† Not determined.

recombinants by this route. As can be seen from Table 4, TK⁻ recombinant virus derived either from WR or Wyeth strains of vaccinia virus invoked a level of neutralizing antibody similar to that detected in the WT infection (variations of less than fourfold are not significant). Recombinant WR-vHBs4 and Wyeth-v55 contain an identical DNA insert coding for HBsAg, and induced similar levels of anti-vaccinia neutralizing antibody (Table 4) and anti-HBsAg antibody (data not shown). When the amount of infectious WR-WT and TK⁻ recombinant viruses, vInfl and v52, present at the site of administration was measured directly by plaque assay, comparable levels of virus were detected between 2 and 10 days post-inoculation. By 42 days, both TK⁻ recombinant and WT virus had been cleared from the primary lesion²⁵. The only difference noted between the TK⁻ recombinant and WT viruses was the delayed appearance of the primary lesion in the TK⁻ recombinant inoculations. By this route, both recombinant virus and WT virus have a low level of virulence for mice.

Reversion of TK⁻ to TK⁺ virus, which readily occurs with point mutations, is unlikely when large insertions are made into the body of the tk gene. In agreement with this expectation, no revertants to TK⁺ virus have been detected (by screening for virus plaques in hypoxanthine/aminopterin/thymidine medium⁴ either after extensive tissue culture passage or in tail tissue isolated from BALB/cByJ mice 8 days after tail scarification with Wyeth-v55. In addition, those recombinants used in this study stably express the foreign genes, as judged by the observation that all screened plaques bind antibody to expressed gene product^{10,12}.

Thus, inactivation of the tk gene of vaccinia virus by the insertion of foreign genes dramatically affects the pathogenesis of vaccinia virus by the i.c. and i.p. routes of inoculation in the mouse. However, by i.d. scarification, which is the preferred route for vaccination, TK⁻ recombinant viruses replicate locally to a level similar to that of WT virus. Our results suggest that TK⁻ recombinant virus has decreased ability to disseminate to, and/or replicate in the internal organs and brain. Thus, by the i.d. scarification route of inoculation, the TK⁻ phenotype may alter the virus pathogenesis in such a manner as to reduce the likelihood of vaccinia-associated complications, such as post-vaccinial encephalitis^{26–28}. One group²⁹ has reported that in 23 out of 40 children diagnosed with postvaccinial encephalitis, vaccinia virus could be detected in the blood, cerebrospinal fluid and pharyngeal secretions. Thus, any genetic modifications to the vaccinia virus that reduce its ability to initiate and maintain a viraemia may potentially lessen the frequency of this most undesirable vaccination-associated complication.

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The cellular oncogene p53 can be activated by mutagenesis

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p53 is a cellular phosphoprotein of short half-life ($t_{1/2}$) which is present at elevated levels in cells transformed by various stimuli including viruses, chemicals and radiation¹⁻⁵. p53 forms specific stable complexes with simian virus 40 (SV40) large-T antigen and an adenovirus E1b protein of relative molecular mass (M_r) 57,000 (refs 4-7). A number of reports have associated p53 with cell proliferation⁸⁻¹¹, and p53 complementary DNA expression constructs immortalize primary cells *in vitro*¹² and render them sensitive to transformation by an activated *ras* oncogene¹²⁻¹⁴. We have examined the biological properties of a set of p53 expression constructs, and report here that cellular immortalization by a wild-type p53 cDNA gene is conditional upon the promoter/enhancer construction used, but that p53 can extend cellular lifespan by a second distinct mechanism involving rearrangements of the coding sequence which give rise to stable protein products. Cells immortalized by one of these mutants are refractory to subsequent transformation by a *ras* oncogene, indicating that cellular immortalization and *ras* cooperation are separate activities.

We constructed p53 expression vectors in which a common cDNA sequence encoding the complete p53 protein was placed under the control of either the Rous sarcoma virus long terminal repeat (RSV LTR) or the SV40 early region promoter (Fig. 1). These constructs were co-transfected with pSV2-Neo into WAXI cells as described elsewhere¹². At 30 h post-transfection, the cells were transferred to medium containing the antibiotic G418.

After 18 days' incubation, colonies of proliferating cells were visible in dishes that had received RSV 53 (Fig. 1). Cultures transfected with pSV53-C or pSV2-Neo reproducibly failed to exhibit any proliferating cells when observed for up to 40 days. In transient expression assays in WAXI and CV-1 cells, however, both RSV 53 and pSV53-C directed the synthesis of mouse p53 protein (see below and Table 1). As the p53 coding sequences are identical in these two constructs, we conclude that the extension of cellular lifespan in this assay is dependent on the use of the RSV LTR as a promoter. In a parallel set of experiments, we placed the wild-type p53 cDNA under mouse mammary tumour virus LTR control. Once again, wild-type p53

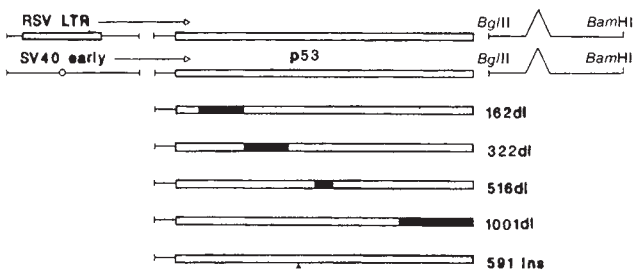


Fig. 1 Schematic diagram of mutant p53 cDNA genes. The murine p53 cDNA gene from plasmid pP53-5 (ref. 21) was used to construct all mutants. 162dl contains a 157-base pair (bp) deletion spanning nucleotides 163-320. The reading frame is conserved by the insertion of an 8-mer *Xba*I linker. 322dl contains a 129-bp deletion spanning nucleotides 322-451. 516dl contains a 50-bp deletion spanning nucleotides 518-568. The reading frame is conserved by insertion of a 10-mer *Eco*RI linker. 1001dl contains a deletion from nucleotide 1,001 to the end of the coding sequence. 591 Ins contains an *Eco*RI 12-mer linker insertion at the corresponding site. All constructs share a common 122-bp 5'-noncoding leader and 3'-noncoding region containing splice and polyadenylation signals from pSV2-Neo as we describe elsewhere¹². , Coding sequence; , deletion; , insertion.

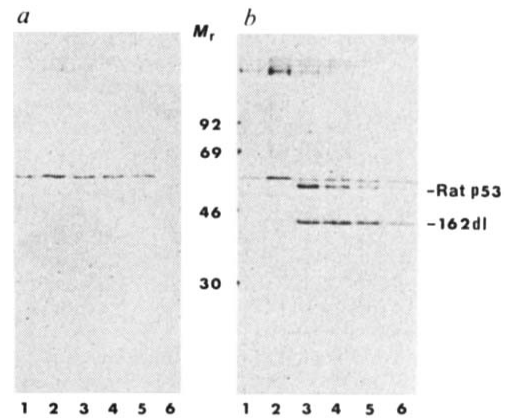


Fig. 2 Pulse-chase labelling of p4JJ WAXI (a) and 162dl WAXI (b). Lanes 1, T_0 immunoprecipitated with pAB419 (ref. 22) (anti-SV40 large-T); lanes 2, T_0 RA3 2C2 (ref. 23) (anti-mouse p53); lanes 3, T_0 pAB421 (ref. 22) (anti-mouse/rat p53); lanes 4, T_{4h} pAB421; lanes 5, T_{8h} pAB 421; lanes 6, T_{24h} pAB421. Values for M_r are given $\times 10^{-3}$.

Methods. 60-mm dishes containing 10^6 cells were grown for 1 h in methionine-free medium +2% FCS. Cells were labelled for 4 h in the same medium +50 μ Ci 35 S-methionine (Amersham) per ml (100 μ Ci per dish). They were then washed twice with DMEM +10% FCS and either lysed (T_0) or cultured at 37 °C and lysed at the appropriate time point. Cells were lysed in modified RIPA buffer (2 ml per dish) and the lysates clarified by spinning for 30 min at 35,000 r.p.m. in a Sorvall TFF 65.13 rotor. Lysates were stored frozen at -70 °C. Immunoprecipitations: lysates were pre-cleared by incubating (1 ml per immunoprecipitation) for 30 min with 10% (v/v) of a 10% suspension of formalin-fixed *Staphylococcus A* cells (BRL Immunoprecipitin) followed by a 5-min centrifugation in a microfuge (MSE Microcentaur) at 4 °C. Each sample was then incubated overnight with 50 μ l of the appropriate hybridoma supernatant at 4 °C, then with 25 μ l of 10% *Staphylococcus A*. Immunoprecipitates were washed three times with modified RIPA buffer before loading onto a 12% SDS-acrylamide gel as described by Laemmli. The gel was fixed in 46% methanol, 7% acetic acid and treated with Enlightening (NEN) before drying and exposure to Kodak XAR film at -70 °C.

constructs alone failed to extend cellular lifespan, but when co-transfected with pT24-Ha-ras-1, which contains an activated Harvey (Ha) *ras* gene, transformed foci of cells with extended lifespan were observed whose growth was conditional on the presence of dexamethasone (P.C. and J.R.J., unpublished). These results are directly comparable to those of other groups^{13,14}, who found that their p53 constructs failed to immortalize cells when transfected alone but gave transformed foci when co-transfected with *ras*.

We constructed mutant p53 cDNA sequences, transferred them into the pSV-C vector (Fig. 1) and tested their ability to extend the *in vitro* lifespan of WAXI cells. By day 18, colonies of proliferating cells were visible in cultures that had been transfected with some of the mutant constructs but again were not induced by pSV53-C even after 28 days (Table 1). Transfection into rat embryo fibroblasts (REF) gave similar results (Table 1). The only differences between these mutant constructs and the parent pSV53-C are defined lesions in the sequences encoding the p53 protein (Fig. 1); the elements involved in transcription and splicing remain unaltered. We conclude that alterations in the coding sequence can enhance the ability of p53 to extend the proliferative potential of mortal cells. We then transfected wild-type and a range of mutant p53 constructs along with pSV2-Neo into WAXI cells and attempted to establish long-term G418-resistant cultures. At day 28, dishes containing foci were treated with trypsin and the cells transferred to 75-cm² flasks. Table 1b shows the results.

Plasmid p4JJKan (ref. 12), encoding wild-type p53 under RSV LTR control and carrying the G418 resistance marker from pSV2-Neo, and one mutant under the SV40 early promoter, gave rise to long-term G418-resistant cultures with no evidence of

Table 1 Effects of p53 expression vectors on growth of WAXI cells

Expt a	Plasmid	Foci per μg per 10^6 cells		
		WAXI	REF	
	PSV2-Neo	0	0	0
	RSV53 + pSV2-Neo	14	21	4
	pSV53-C + pSV2-Neo	0	0	0
	pSV-162dl + pSV2-Neo	15	45	16
	pSV-591 Ins + pSV2-Neo	70	40	30

Expt b	Plasmid	Foci per μg per 10^6 cells	Continued growth	Transient expression in WAXI cells		
				Alone	+SV40 wild-type	+SV40 D10T
	pSV2-Neo	0	—	—	—	—
	p4JJKan	13	+	—	+	+
	RSV53					
	+pSV2-Neo	10	—	—	+	+
	pSV53-C					
	+pSV2-Neo	0	—	—	+	+
	pSV-162dl					
	+pSV2-Neo	14	+	+	+	+
	pSV-322dl					
	+pSV2-Neo	3	—	+	+	+
	pSV-591 Ins					
	+pSV2-Neo	7	—	+	+	+
	pSV-516dl					
	+pSV2-Neo	8	—	+	+	+
	pSV-1001dl					
	+pSV2-Neo	0	—	—	+	+

Expt a, transfection of WAXI cells by p53 expression vectors as resistant foci after treatment with G418 30 h post-transfection. Subconfluent 60-mm dishes of WAXI cells cultured in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal calf serum (FCS) were transfected with plasmid DNA at $5 \mu\text{g}$ per dish using the calcium phosphate co-precipitation method as we describe elsewhere¹². At 30 h post-transfection, cells were transferred to medium containing $400 \mu\text{g ml}^{-1}$ Geneticin (G418). Selection was maintained for up to 40 days with medium changes every 3 days. To assay for long-term growth, mass cultures from transfected 60-mm dishes were trypsinized, transferred to 75-cm² flasks and maintained in selective medium. Expt b, Transient proliferation compared with long-term growth of WAXI cells transfected with p53 expression constructs. Foci of proliferating cells were counted at 28 days. Transient expression assays were carried out by DEAE-dextran transfection. Subconfluent WAXI cells were trypsinized, resuspended in DMEM + 10% FCS, seeded into 60-mm dishes and allowed to adhere for 2 h at 37°C. They were then washed twice with DMEM, and 1.5 ml of a solution containing $5 \mu\text{g}$ plasmid DNA, $200 \mu\text{g ml}^{-1}$ DEAE-dextran (Sigma, M_r 500,000) in DMEM was added. Dishes were incubated for 4 h at 37°C, the DNA solution was then aspirated, and replaced by 1 ml of Hank's buffered saline containing 10% dimethyl sulphoxide (Fisons). Dishes were incubated for 2 min at 37°C, the solution aspirated, washed twice with phosphate-buffered saline, and cells re-fed with DMEM + 10% FCS. At 72 h post-transfection, cells were fixed in methanol/acetone 1:1, and expressed p53 visualized by indirect immunofluorescence, using the mouse-specific monoclonal antibodies pAB246 and pAB248 (J. Yewdell *et al.*, in preparation). Transient expression experiments gave identical results when scored in monkey CV-1 cells.

terminal differentiation or crisis. Colonies derived from other mutants, and from wild-type p53 under RSV LTR control alone failed to become established, and the cells exhibited a typical senescent morphology. Thus, these constructs induced a limited increase in proliferative potential whereas the sequences from pSV2-Neo, which include the SV40 origin of replication and the early region promoter and enhancer, exert a *cis*-acting effect that permits the indefinite growth of WAXI cells transfected by p4JJKan. This requirement in *cis* may explain why other groups^{13,14} were unable to isolate cell lines immortalized by p53.

Transient expression assays were carried out in WAXI and CV-1 cells. Mouse p53 was not detectable by immunofluorescence in cultures transfected with p4JJKan, RSV 53 or pSV53-

C (Table 1b). However, p53 was clearly visible in cultures transfected with pSV-162dl and other mutants (Table 1b). Mouse p53 was also readily detectable by immunofluorescence in long-term cultures derived from pSV-162dl transfection but not in those from p4JJKan (data not shown). Co-transfection of p4JJKan, RSV 53 or pSV53-C with constructs encoding either wild-type SV40 large-T antigen or the replication-defective mutant D10 (ref. 15) produced high levels of mouse p53 in recipient cells (Table 1b), demonstrating that these constructs direct the synthesis of p53, but that the accumulation of detectable levels of wild-type p53 in these assays requires the presence of a stabilizing SV40 large-T antigen. Long-term mass cultures of WAXI cells immortalized by either p4JJKan (p4JJ WAXI) or pSV-162dl (162dl WAXI) were grown up and the $t_{1/2}$ of the expressed p53 was measured by pulse-chase labelling. The results (Fig. 2) show that the pSV-162dl gene product, which migrates with an apparent M_r of $\sim 43,000$, has a $t_{1/2}$ of ~ 24 h. Interestingly, the $t_{1/2}$ of endogenous rat p53 was ~ 4 h whereas we were barely able to detect p53 in p4JJ WAXI cells. In each case the ability of mutant p53 constructs to extend the lifespan of mortal cells *in vitro* was associated with a reduction in the sensitivity of p53 to the cellular mechanisms controlling its half-life in untransformed cells. These results demonstrate that p53 can be activated by mutational changes in the cellular gene and suggest that mutation leading to increased stability of a short- $t_{1/2}$ protein is a novel mechanism by which a cellular oncogene can participate in multi-stage carcinogenesis. An additional point concerns the structure of the lesions that enhance p53 activity. We, along with several other groups, are currently screening human tumour material for evidence of p53 gene rearrangements. The results presented here demonstrate that p53 can be activated by mutations that would be undetectable by Southern blotting and suggest that fine-structure S_1 -nuclease mapping may be required to detect alterations in the cellular gene resulting in abnormal function.

p4JJ WAXI and 162dl WAXI mass cultures were transfected with cloned activated c-Ha-ras using pT24-Ha-ras-1. As we had shown previously¹², the activated *ras* gene efficiently transformed WAXI cells immortalized by wild-type p53. However, in repeated experiments no foci were induced in any 162dl WAXI mass cultures. These cells are not refractory to transfection, as we can isolate SVGPT/*ras* co-transfectants that show no morphological evidence of transformation.

Several reports¹⁶⁻¹⁸ have suggested that the malignant transformation of primary cells *in vitro* requires at least two distinct events. The first has been defined as cellular immortalization or establishment¹⁶⁻¹⁹ and can be induced by chemical carcinogens²⁰ or by transfection with one of several cloned oncogenes such as adenovirus early region 1A (Ela; ref. 17) or the avian myelocytomatosis oncogene *v-myc* (ref. 18). Cells immortalized by such reagents can subsequently be fully transformed by oncogenes of a second complementation group such as *ras* and adenovirus E1b. In the case of p53, however, our data demonstrate that cellular immortality itself is insufficient to permit transformation by a *ras* gene, and that cellular immortalization and *ras* cooperation by p53 are distinct activities that can be resolved by mutagenesis.

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Positive identification of an immigration test-case using human DNA fingerprints

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The human genome contains a set of minisatellites, each of which consists of tandem repeats of a DNA segment containing the 'core' sequence, a putative recombination signal in human DNA¹. Multi-allelic variation in the number of tandem repeats occurs at many of these minisatellite loci. Hybridization probes consisting of tandem repeats of the core sequence detect many hypervariable minisatellites simultaneously in human DNA¹, to produce a DNA fingerprint that is completely individual-specific and shows somatic and germline stability². These DNA fingerprints are derived from a large number of highly informative dispersed autosomal loci and are suitable for linkage analysis in man³, and for individual identification in, for example, forensic science and paternity testing². They can also be used to resolve immigration disputes arising from lack of proof of family relationships. To illustrate the potential for positive or inclusive identification, we now describe the DNA fingerprint analysis of an immigration case, the resolution of which would have been very difficult and laborious using currently available single-locus genetic markers.

The case concerned a Ghanaian boy born in the United Kingdom who emigrated to Ghana to join his father and subsequently returned alone to the United Kingdom to be reunited with his mother, brother and two sisters. However, there was evidence to suggest that a substitution might have occurred, either for an unrelated boy, or for a son of a sister of the mother; she has several sisters, all of whom live in Ghana. As a result, the returning boy was not granted residence in the United Kingdom. Analysis of conventional genetic markers (ABO, Rh, MN, Se, Pi, Lu, K, Fy, Jk, Gm, Hp, EAP, GLO, PGM, Gc, EsD and HLA; K.L.I. Rogers, personal communication) showed that the woman and boy in dispute were almost certainly related (probability of no relationship = 0.01), but could not determine whether the woman was the boy's mother or aunt. At the request of the family's solicitor, we therefore carried out a DNA fingerprint analysis to determine the maternity of the boy. To complicate matters, neither the father nor any of the mother's sisters was available for analysis. Furthermore, while the mother was certain that the boy was her son, she was not sure about his paternity. DNA fingerprints from blood DNA samples taken from available members of the family (the mother M, brother B, sisters S1 and S2 and the boy X in dispute) were prepared by Southern blot hybridization to two minisatellite probes, each of which detects a different set of hypervariable minisatellites in human DNA^{2,3} (Fig. 1).

The first step was to establish the paternity of X from the patterns of hypervariable fragments. Although the father was unavailable, most of his DNA fingerprint could be reconstructed from paternal-specific DNA fragments present in at least one of the three undisputed sibs (B, S1, S2) but absent from M. Of

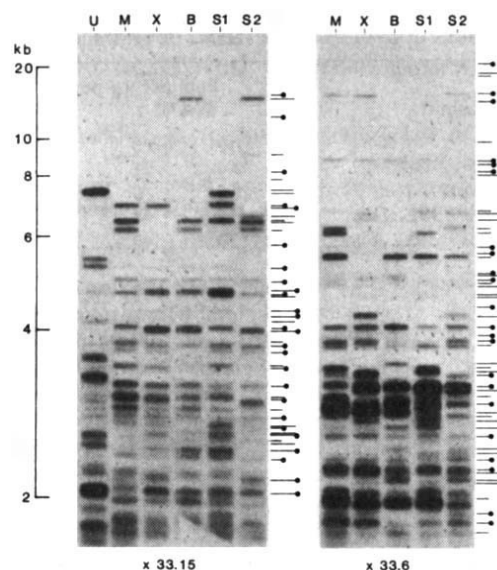


Fig. 1 DNA fingerprints of a Ghanaian family involved in an immigration dispute. Samples (8 µg) of blood DNA from the mother (M), the boy in dispute (X), his brother (B), sisters (S1, S2) and an unrelated individual (U) were digested with *Hinf*I, electrophoresed through a 0.7% agarose gel and Southern blot-hybridized to ³²P-labelled minisatellite probe 33.6 or 33.15 as described elsewhere^{1,2}. Fragments present in the mother's (M) DNA fingerprints are indicated by a short horizontal line; paternal fragments absent from M but present in at least one of the undisputed sibs (B, S1, S2) are marked with a long line. 'Maternal' and paternal fragments transmitted to X are shown by a dot. The DNA fingerprints of X contain no additional resolved fragments. All fragments including fainter fragments not visible in this photograph were scored from the original autoradiographs taken at various exposures; partially resolved fainter bands, particularly towards the bottom of the gel, which could not be reliably scored were ignored. kb, kilobases.

Methods. Quantitation of DNA fingerprints: 61 DNA fragments were scored in M, compared with 39 fragments inherited specifically from the father. One-eighth of the father's heterozygous DNA fragments will not be transmitted to B, S1 or S2 and thus the corrected estimate for the number of paternal-specific fragments is $39 \times 8/7 = 45$. Since the total number of fragments in the DNA fingerprints of M and the father should be approximately equal, the number of fragments in M which are shared by the father is $\sim(61 - 45) = 16$. The mean probability of band sharing (x) in M and the father is therefore $16/61 = 0.26$, consistent with previous estimates derived from screening a random sample of Northern Europeans ($x = 0.2$, ref. 2). Approximately half of the 45 paternal bands were transmitted to B, S1 and S2 (18, 24 and 18, respectively), as expected for heterozygous bands. Of the 61 bands in M, more than half were inherited by B, S1 and S2 (32, 38 and 39, respectively; mean = 36.3), as expected since some of M's bands will be shared by the father and will therefore be transmitted to most or all children. If M's DNA fingerprints contain n shared bands transmitted to all children plus $(61 - n)$ heterozygous bands transmitted to half the children, then $n + 0.5(61 - n) = 36.3$, whence $n = 12$, consistent with the estimate of ~ 16 bands common to M and the father (see above). The DNA fingerprints of X are comprised of 21 paternal-specific fragments plus 40 bands shared with M. The proportion of the latter bands which are maternal-specific and not shared by the father can be estimated in two ways. First, the number of maternal-specific bands should be roughly equal to the number specific to the father, that is, $45/2 = 22.5$. Second, n (~ 12) of the 40 'maternal' bands in X will be shared maternal/paternal bands (see above), which leaves 28 maternal-specific bands in X. The number of fragments that X has acquired specifically from his mother is therefore ~ 25 . Probabilities of band sharing: The mean probability that a fragment in one individual is matched by a band of similar electrophoretic mobility and autoradiograph intensity in a second random person is defined as x ($x = 0.2-0.26$, see above). Larger minisatellite fragments are less frequently shared, probably due to lower allele frequencies and better electrophoretic resolution, and thus the fragment sharing probability x is heterogeneous². Since almost all fragments are inherited independently³, the maximum probability that all n fragments in an individual are present in a second random individual is therefore x^n ; any heterogeneity in x will reduce this probability. Band sharing between sibs: If shared bands always represent identical alleles of the same hypervariable locus, then, assuming that all alleles have equal frequencies, x is related to the allele frequency q by $x = 2q - q^2$. At Hardy-Weinberg equilibrium, the probability that a band in an individual is also present in a sib can be shown to be $(4 + 5q - 6q^2 + q^3)/4(2 - q)$ (case i). The other extreme case is that bands shared by unrelated individuals are never allelic (that is, that there are many loci at which a band with a given electrophoretic mobility may be found). Then q can be defined as the probability that a given band will be found in a random gamete from the population. As before, $x = 2q - q^2$, but the probability of band sharing between sibs can now be shown to be $(1 + q - q^2)/(2 - q)$ (case ii). For $x = 0.26$, q is 0.14 and the probability of band sharing is 0.62 (case i) or 0.60 (case ii).

the 39 paternal fragments so identified, approximately half were present in the DNA fingerprints of X (Fig. 1). Since DNA fragments are seldom shared between the DNA fingerprints of unrelated individuals² (see individual U in Fig. 1), this suggests very strongly that X has the same father as B, S1 and S2. After subtracting these paternal-specific DNA fragments, there remained 40 fragments in X, all of which were present in M. This in turn provides strong evidence that M is the mother of X, and therefore that X, B, S1 and S2 are true sibs.

How certain is this identification? We have shown previously that the mean probability that a fragment in the DNA fingerprint of one person is present in a second individual selected at random is approximately 0.2 for Northern Europeans². The corresponding estimate for the father and M is ~ 0.26 (Fig. 1 legend), establishing that DNA fingerprint variability in these Ghanaians is not significantly different from that of Northern Europeans. In the following probability estimates, we shall make the highly conservative assumption that all bands are shared with a uniform probability of 0.26 per band (see Fig. 1 legend for further discussion).

The first question is whether X is related to this family. The DNA fingerprints of X contain 61 scorable fragments, all of which are present in M and/or the father. If X is unrelated, the probability that each of his bands is present in these parents is $1 - (1 - 0.26)^2 = 0.45$; the probability that M and/or the father by chance possess all 61 of X's bands is therefore $0.45^{61} = 7 \times 10^{-22}$. X is clearly related to this family.

The next problem is whether an unrelated woman, and not M, could be the mother of X. The DNA fingerprints of X contain 40 'maternal' fragments, of which we estimate that ~ 25 were inherited specifically from the mother; remaining fragments are shared between the mother and father and cannot therefore be used to adduce evidence for M's maternity (Fig. 1 legend). All 25 maternal-specific fragments in X are present in M. The chance that M is unrelated to X but happens to share all 25 fragments is therefore $0.26^{25} = 2 \times 10^{-15}$. Thus, X and M must be related.

The final and most difficult problem is whether any of M's sisters, who were not available for analysis, could be the mother of X (the father, of course, would have to be M's husband). If bands are shared between random people with a mean probability of 0.26, the corresponding chance that a fragment in one individual is also present in a sib is 0.62 (Fig. 1 legend). The odds that M is a sister of X's true mother but by chance contains all 25 of X's maternal-specific bands are therefore $0.62^{25} = 6 \times 10^{-6}$. We therefore conclude that, beyond any reasonable doubt, M must be the true mother of X. This evidence, along with results from conventional marker analysis, was provided to the immigration authorities, who dropped the case against X and granted him residence in the United Kingdom, allowing him to remain with his family.

This difficult case demonstrates how DNA fingerprints can give unequivocal positive evidence of relationship, even in some cases where critical family members are missing. The present case was simplified by the fact that X has the same father as his sibs, and that this father did not transmit any bands solely to X (on average, 1/16th of paternal bands would be so transmitted). Such X-unique fragments, while apparently weakening the evidence for the relationship between X and M, would not in practice necessarily invalidate the analysis. X would be unlikely to have more than 5 such paternal fragments, in addition to the 25 maternal-specific fragments. The odds of at least 25 out of 30 specified bands matching by chance between X and M if they are unrelated, or if M is X's aunt, is 8×10^{-11} and 9×10^{-3} , respectively. This analysis is therefore robust and would give clear evidence for or against claimed relationships in most such cases. Usually, of course, all relevant members of a family will be available, in for example paternity disputes or with families having difficulties in reuniting by immigration; such cases will almost always be resolvable using a single DNA fingerprint test.

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Repeat sequence families derived from mammalian tRNA genes

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Short interspersed repetitive DNA sequences (SINEs) are the major component of dispersed repetitive DNA in all mammalian genomes (see refs 1-6 for reviews). Most SINEs contain an intragenic RNA polymerase III promoter that initiates transcription at the 5' end of the repeated DNA sequence⁷⁻⁹ and which has been proposed to facilitate the transposition and amplification of these sequences by an RNA-intermediate mechanism^{10,11}. We have discovered several SINE families in the prosimian *Galago crassicaudatus* which have promoter regions similar to transfer RNA genes^{12,13}. To determine the relationship between *Galago* SINEs and mammalian tRNA genes, we have compared their sequences. Here, we demonstrate that the *Galago* monomer and type II SINE families¹² are 68 and 62% homologous, respectively, with a human methionine tRNA gene¹⁴. We have extended our analysis to include the rat identifier¹⁵ and mouse B2 (ref. 16) families and show that their sequences are closely related to alanine and serine tRNA genes, respectively. Our observations suggest that many mammalian SINE families are amplified tRNA pseudogenes.

The most extensively characterized family of SINEs is the human *Alu* family, present in excess of 500,000 copies in the haploid human genome¹⁷. The *Alu* family consensus sequence corresponds to a dimeric structure composed of two related and tandemly arranged monomeric units, each ~ 130 base pairs (bp) long with an additional 31 bp in the middle of the right-half sequence (Fig. 1)¹⁸. This dimeric structure is conserved throughout the primates^{13,19}, but *Alu* family-related sequences are found only as monomeric sequences in rodent species^{1,2,20-22}. This difference in primate and rodent *Alu* family structure suggests that there has been a rapid amplification or replacement of *Alu* family sequences since the rodent-primate divergence.

To determine the mechanisms involved in generating or altering SINEs, we have studied the evolution of *Alu* family structure in several primates and discovered that *G. crassicaudatus* contains two types of *Alu* family sequence (Fig. 1). One is closely related to the human *Alu* family (type I)¹³ and the other (type II)¹² has an identical right half, but a very different left-half sequence. We have also discovered that the *Galago* genome contains an independent repetitive DNA family, designated 'monomer', which is not homologous with the human *Alu* family¹². The monomer family is similar in copy number to the other two *Galago* families and is closely related to the left-half sequence of the type II family (Fig. 1). The structural relationship of these *Galago* families suggests that the type II sequence has arisen by the integration of a monomer family member into the centre of a type I family¹². This fusion of two separate repeats has apparently spread as an independent family using the promoter supplied by the monomer sequence.

We have shown previously that the RNA polymerase III promoter sequence (block A and B) for the type II family¹² and, therefore, also the monomer, is closely homologous with the tRNA promoter consensus sequence²³. This similarity between the monomer and tRNA promoter sequences, together with the observation of some capability for intramolecular base-pairing within potential monomer family RNA transcripts, suggested the possibility of a tRNA ancestry for the monomer family.

To determine the validity of this proposed ancestry, we sequenced 17 monomer family members and built a consensus sequence for this family (data not shown). We also compared our monomer consensus sequence with all the mammalian tRNA genes listed in GenBank and in the published literature (Fig. 2). From this analysis, we found that most of the tRNA genes contained regions of homology with the block A and B promoter sequences of the monomer family. Strikingly, however, a series of methionine tRNA genes from different mammalian species showed very strong homology with the monomer consensus sequence. The best homology (~68%) was found with the human methionine tRNA gene¹⁴ (Fig. 2a). Unlike homologies with other tRNA genes, this match occurred throughout the entire length of the tRNA sequence and had only slightly better homology within the promoter consensus sequences than with the rest of the sequence.

Figure 3 shows the classical cloverleaf structure of the human methionine tRNA (a) and the analogous folding of the monomer family consensus sequence (b). The base-pairing of the dihydrouridine stem, the anticodon stem and the pseudouridine stem in the monomer sequence coincides almost perfectly with the tRNA structure (Fig. 3b). The aminoacyl stem of the monomer base-pairs less perfectly, with several mismatches and a one-base loop-out on one strand. There is also an insertion of two bases in the dihydrouridine loop of the monomer consensus sequence. This is consistent with tRNA structure, however, as this loop typically varies from seven to nine bases in different tRNAs²⁴. The extra bases at both the 5' and 3' ends of the monomer consensus are consistent with tRNA structure in that the transcriptional unit for many tRNA genes includes extra bases at both ends, which are removed by post-transcriptional processing. In addition, the monomer consensus sequence contains many other features normally conserved in tRNA structure, including the methionine tRNA anticodon CAU and several highly conserved nucleotide positions involved in folding of the tRNA structure²⁵.

Individual monomer family sequences do not usually fit the methionine tRNA structure as well as the consensus sequence (data not shown). In several examples, many mutations weaken the homologies of individual members with the tRNA structure and there is no evidence for compensating changes in an individual monomer sequence which maintain intramolecular

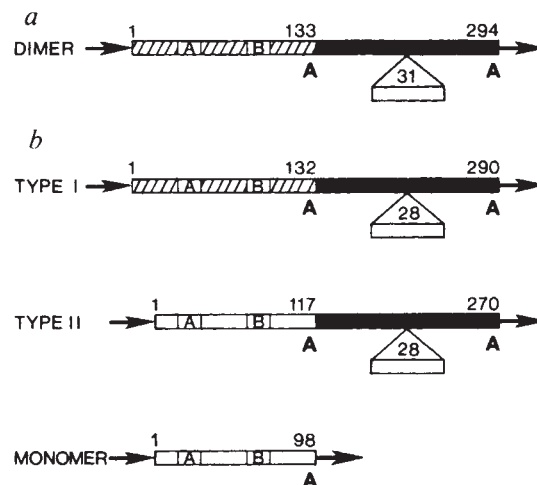
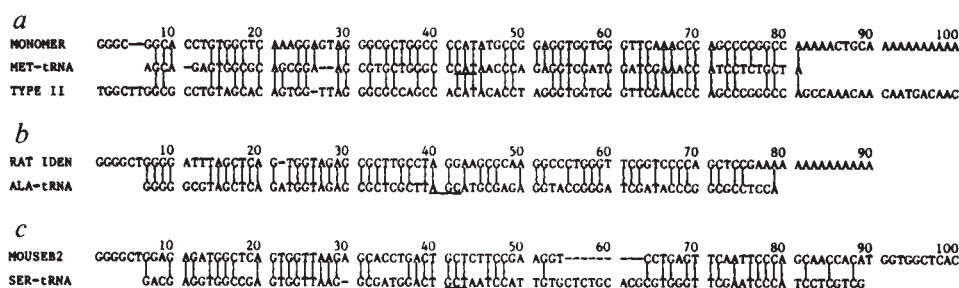


Fig. 1 Structural relationship of the human *Alu* family (a) to three major repetitive DNA sequences found in *Galago* (b). The consensus structure of the human *Alu* family and the *Galago* type I, type II and monomer families are shown schematically to demonstrate regions of homology (depicted by identical shading) among primate repetitive DNA sequences. The human *Alu* family (dimer) is composed of two related and tandemly arranged sequences (shaded and solid boxes) with an additional 31 bases in the right-half sequence¹⁸. The *Galago* type I family is closely related to the human *Alu* family and demonstrates sequence homology to the human dimer throughout its structure¹³. The type II family in *Galago* has a right-half sequence (solid box) homologous with the type I and human *Alu* right-half sequences¹². However, the left-half region of the type II family (open box) is appreciably different from analogous regions in the type I and human *Alu* families. The *Galago* monomer family (open box) is closely related to the type II left-half sequence. Some common features shared by all four primate repetitive DNA families are the direct repeats (arrows) that flank the repetitive sequences, the intragenic RNA polymerase III promoters (block A and B), and the oligo-dA-rich sequences located at the 3' end of each region (marked by an A beneath the region).

base-pairing. Thus, we do not believe that the tRNA-like structure is under a strong evolutionary constraint in the monomer family, but is probably a vestigial remnant of its tRNA progenitor.

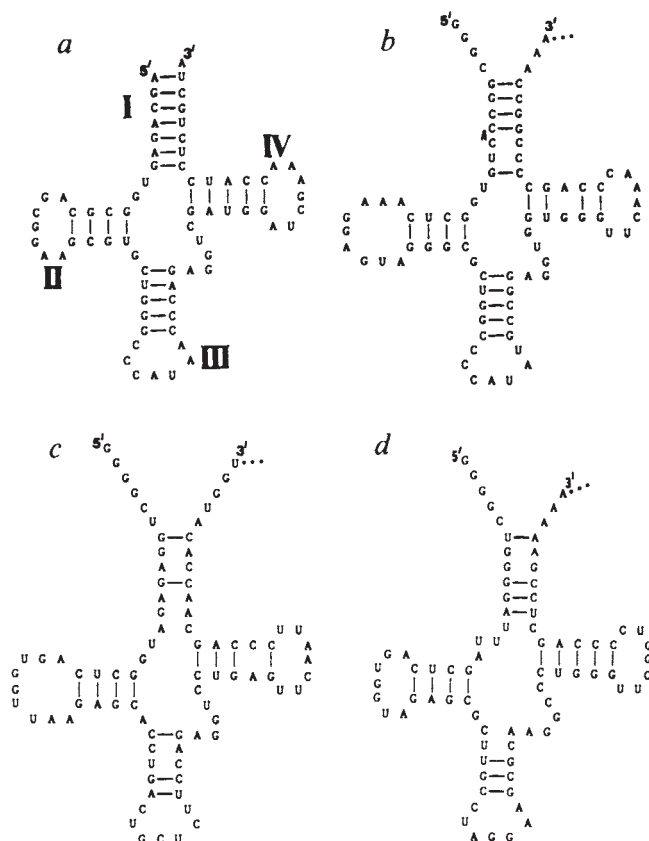
The possibility that other highly repetitive DNA families are diverged from RNA polymerase III transcribed genes was suggested by the observations that the *Alu* family seems to have evolved from a 7S RNA gene²⁶ and that the monomer family is apparently derived from a methionine tRNA gene. Therefore, we compared two other major repetitive DNA families, the

Fig. 2 Sequence homology among mammalian repetitive DNA sequences and tRNA genes. Sequence homologies to all tRNA genes were determined by comparing individually the consensus sequences for the *Galago* monomer, *Galago* type II, rat identifier and mouse B2 families to several non-mammalian and all mammalian tRNA genes listed in GenBank and in the published literature³⁵. In each case the tRNA gene with the best homology to a particular mammalian repetitive



DNA sequence is aligned with it to show similarities in the sequence. a, *Galago* monomer and type II *Alu* family consensus sequences compared with the human methionine tRNA gene¹⁴ to demonstrate regions of homology. Vertical lines show the positions of homologous bases in each sequence. The anticodon for the methionine tRNA gene (CAT) is underlined to indicate its position within the sequence. Deletions are represented by dashes in the sequence. We have shown only the first 100 bases of the type II consensus sequence as this is the only region homologous to the monomer and methionine tRNA gene sequences. b, Sequence comparison of the rat identifier sequence¹⁵ and the *B. mori* alanine tRNA gene²⁸. The anticodon sequence for the alanine tRNA gene (AGC) is underlined to indicate its position. c, Comparison of sequences for the mouse B2 family¹⁶ and the rat serine tRNA gene²⁹. The anticodon sequence for the rat serine tRNA gene (GCT) is underlined. Dashes in either sequence represent a deletion relative to the other sequence. We have shown only the first 100 bases of the mouse B2 sequence as this is the only region homologous with the Ser tRNA gene.

Fig. 3 Secondary structure comparisons of the human methionine tRNA to putative transcripts of several mammalian repetitive DNA sequences. The human methionine tRNA (*a*) is drawn in a typical cloverleaf structure to demonstrate the base-pairing of its stems. The 5' and 3' termini are labelled, as are the aminoacyl stem (I), the dihydrouridine loop (II), the anticodon loop (III), and the pseudouridine loop (IV). Using the homologies depicted in Fig. 2, we have drawn the monomer consensus (*b*), a portion of the mouse B2 family consensus (*c*) and the rat identifier family consensus (*d*) sequences as cloverleaf tRNA-like structures. Lines are drawn between bases in the tRNA structures to demonstrate regions of base-pairing. Guanine-uridine base pairs are allowed in this analysis of secondary structure.



mouse B2 family¹⁶ and the rat identifier family¹⁵, with the mammalian tRNA genes in GenBank. In both cases, significant homologies are found.

We originally compared the rat identifier family (ID) with all mammalian tRNA genes and found that 64% of its sequence is homologous with the human phenylalanine tRNA²⁷. However, subsequent comparisons with tRNA genes that have not been characterized in mammals demonstrated that there is a stronger homology (73%) to an Ala tRNA gene isolated from *Bombyx mori*²⁸ (Fig. 2*b*). The homology with the silkworm tRNA gene is evenly distributed throughout the length of the sequence and the Ala tRNA anticodon, AGC (see Figs 2*b*, 3*d*), closely resembles that of the rat identifier sequence (AGG). For these reasons we propose that the rat identifier family is derived from an Ala tRNA gene. Although we cannot exclude a similar tRNA gene as the actual progenitor, the 73% overall homology between these sequences is the best we have encountered when comparing SINE families with tRNA genes.

The mouse B2 family homology with the rat serine tRNA gene²⁹ (Fig. 2*c*) is again consistent with selecting the tRNA gene with the best homology (64%) and the proper anticodon sequence (GCT for Ser). The major difference between these sequences is that several bases are missing around position 60 in the mouse B2 consensus sequence. This region corresponds to the small, looped-out region between the anticodon stem and the pseudouridine stem (Fig. 3*c*). It is common for variation in sequence length to occur in this region of different tRNA genes, which suggests that the mouse B2 family has arisen from a different Ser or closely related tRNA gene and not the one actually used in this comparison.

Several structural features of the SINEs are thought to be important for the mechanism of amplification through an RNA intermediate^{10,11}. The discovery that tRNA genes are progenitors of repetitive DNA families helps us to understand the factors that allow them to amplify so effectively. An obvious requirement is for a RNA polymerase III promoter to direct the formation of RNA transcripts. Because the promoter is internal, the transposed sequences may also be capable of transcription. Second, there should be a region of oligo-dA sequence near the 3' end

of the SINEs to allow efficient self-priming for reverse transcription by the uridine-rich sequence at the 3' end of the transcript. This dA-rich sequence could be acquired either by genomic sequence rearrangements or by transposition of a tRNA gene or pseudogene to an oligo-dA region. Third, tRNA-related genes cannot contain sequences within their structure that block the efficient reverse transcription of the RNA, such as very strong secondary structures³⁰. In this regard, the SINE families depicted in Fig. 3 show less secondary structure than the parent genes and might therefore be expected to reverse-transcribe more efficiently. Fourth, amplification of the tRNA-related sequence should not have any deleterious effects on the cell. Thus, sufficient mutations may have to accumulate in the tRNA sequence to abolish competition with cellular function of the parent gene before extremely high copy numbers can be accommodated by the cells. This could require several point mutations or deletions to alter sufficiently the gene structure.

The finding that many of the major SINE families have evolved from RNA polymerase III genes of known function suggests that most of the members of these families are simply pseudogenes with no physiological function. In addition to the tRNA-derived families discussed here, the mouse B1 and primate *Alu* families seem to have originated from the 7SL RNA gene²⁶. Since the submission of this manuscript, tRNA homologies for a goat repetitive DNA family⁶ and for the mouse B2 and rat ID families³¹ have also been found independently. Theoretical arguments for such families as 'selfish DNA' have already been presented^{32,33}. The suggestion that SINEs are pseudogenes is further supported by the relatively recent evolution of some of these families and the presence of each family in only a limited species range. The monomer family is the best illustration of this suggestion, as hybridization to bovine, mouse, tree shrew, lemur, owl monkey, rhesus monkey and human genomic DNA showed no evidence for its amplification outside the *Galago* genome (data not shown). If these repeated DNA families serve some overriding function, such as origins of DNA replication or gene regulation³⁴, then this function must be very evolutionarily adaptable, as the major SINE families seem to be heterogeneous in many different species. This does not

exclude a more generalized function served by the presence of many dispersed RNA polymerase III promoters. Whether a limited group or a subset of each of the SINE families has evolved or taken over some previous cellular function is unknown. The insertion of hundreds of thousands of repetitive DNA elements into new genomic locations over the past 45 Myr must have had major effects on the structure and evolution of the genome.

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Negative control at a distance mediates catabolite repression in yeast

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In prokaryotic organisms, the control of gene expression is mediated by regulatory proteins that activate or repress transcription^{1,2}. However, the molecular mechanisms of positive and negative control are different. In terms of negative control, repressor proteins bind to sites located within the promoter region and as a consequence sterically interfere with functional binding by RNA polymerase. Here, I examine the properties of a regulatory sequence that specifies catabolite (glucose) repression in the yeast *Saccharomyces cerevisiae*. Specifically, a DNA segment containing this regulatory site was fused upstream of the intact *his3* promoter region and structural gene at several locations. Normally, *his3* expression in these derivatives occurs at a basal level which can be induced by conditions of amino-acid starvation. However, in glucose medium, the catabolite regulatory sequence overrides the normal *his3* promoter elements and reduces transcription both in normal and starvation conditions. The implication from these

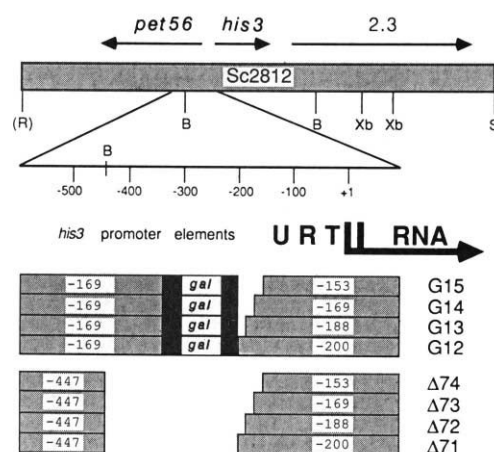


Fig. 1 Experimental design. All the DNA molecules derive from YIp5-Sc2812, which contains a 6.1-kilobase (kb) *EcoRI-SalI his3* segment (shaded bar at top) cloned in the *ura3⁺* integrating vector YIp5 (refs 13, 22). The locations and orientations of the *his3*, *pet56* and 2.3-kb transcripts are shown as arrows above the shaded bar, and restriction sites are indicated by vertical lines: R, mutated *EcoRI* site; B, *BamHI*; Xb, *XbaI*; S, *SalI*. Beneath the bar is an expanded view of the *his3* promoter region, which includes the upstream promoter element (U), the positive regulatory site (R), the TATA promoter element (T), and the RNA initiation sites located at positions +1 and +12 (solid vertical lines)^{13,16}. The derivatives that were tested in the present work (numbered at the right-hand side) were constructed by joining DNA segments containing the *his3* promoter region and structural gene (right side) to upstream segments in which the *gal10* element was either present or absent (left side). The promoter-containing DNA fragments have varying amounts of upstream sequences and they are bounded by *EcoRI* linkers (details of the method to be published elsewhere). The precise endpoints (indicated within the shaded bars and listed in Table 1) were determined by dideoxy DNA sequencing²³ of appropriate fragments cloned into the single-stranded vector mp8 (ref. 24). To create *gal-his3* fusions, the *his3* promoter segments were joined via the *EcoRI* cohesive ends to a segment containing the 365-bp *gal10* region (solid box) and *his3* sequences upstream of nucleotide -169 (ref. 13) (not drawn to scale). The control molecules lack the *gal* region, but they contain *his3* sequences upstream of -447. These derivatives, which effectively have an *EcoRI* site at the normal position of the *BamHI* site, are derived from YIp5-Sc3319 (ref. 13). To assess the phenotypes of the derivatives, DNA molecules were cleaved with *XbaI* and introduced into KY117 (relevant genotype *ura3-52*, *his3-Δ200*, *GAL⁺*) by selecting for *Ura⁺* transformants. As the *his3-Δ200* allele deletes all sequences between -178 and +874 (unpublished results), the only *his3* sequences in the resulting strains arise from integration of the transforming DNA. The 1.7-kb *BamHI*-generated DNA fragment was used as a hybridization probe to prove that the transformants resulted from integration of a single molecule into the *his3* locus; it was also used to measure RNA levels (see Fig. 2).

results is that in contrast to catabolite repression in *Escherichia coli*, which is mediated by catabolite-activating protein (CAP)³, catabolite repression in yeast occurs by a negative control mechanism involving a putative repressor protein. The observation that this regulatory site exerts its repressing effects even when located upstream of an intact promoter region suggests that repression in yeast is not mediated by steric interference between regulatory proteins and the transcriptional apparatus.

Certain yeast genes are transcribed at reduced levels when the sole carbon source in the medium is glucose as opposed to other compounds which are less directly metabolized⁴⁻⁷. This glucose effect (catabolite repression) is usually observed for genes involved in the metabolism of poor carbon sources, the rationale being that such gene products are not needed when cells are metabolizing glucose⁸.

The *gal7*, *gal1* and *gal10* genes, which convert exogenous galactose into glucose 1-phosphate, are highly induced in galactose-containing medium, and they are also subject to catabolite

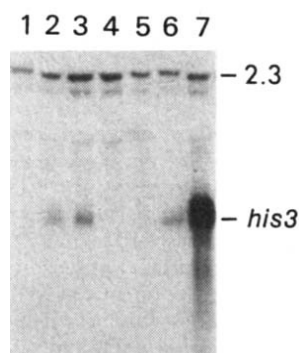


Fig. 2 RNA levels. Strains were grown in YP medium (1% yeast extract, 2% peptone) containing either 2% glucose or 2% galactose as the sole source of carbon. RNA was extracted from half of a 30-ml culture collected in the exponential phase of growth. Lanes 1–6 represent 50 µg total RNA from the following strains grown in glucose medium: the untransformed parent KY117 (lane 1); control molecule, –200 endpoint (lane 2); control molecule, –153 endpoint (lane 3); *gal-his3* fusion, –188 endpoint (lane 4); *gal-his3* fusion, –153 endpoint (lane 5); the wild-type strain KY114 (lane 6). Lane 7 contains 50 µg RNA from a strain with Sc3357 grown in galactose medium. All RNAs were separated electrophoretically in a 1.7% agarose gel containing 6% formaldehyde, then transferred to nitrocellulose. The hybridization probe was ³²P-labelled Sc2676 DNA (corresponding to the 1.7-kb *Bam*HI fragment) prepared by nick-translation; this probe is homologous to the entire *his3* gene, and also to 330 bp of a 2.3-kb mRNA species, which provides an internal control. Details of the method have been described previously¹⁴.

repression⁹. Studies of the divergently transcribed *gal1* and *gal10* genes indicate that the regulatory sites specifying catabolite repression and galactose induction are located several hundred base pairs (bp) upstream from the RNA initiation site^{10–13}. This location is well upstream from the TATA sequences which are necessary, but not sufficient, for transcriptional initiation. Furthermore, when the upstream promoter elements of the *cyc1* and *his3* genes are replaced by a 365-bp fragment from the *gal1,10* region, these unrelated genes are now subject to both catabolite repression and galactose induction^{10,13}.

Note, however, that in these fusions, the *gal* region is fused to DNA segments that by themselves are transcriptionally inactive because they contain only the TATA element. Thus, it is impossible to determine whether the lack of expression in glucose medium is due to a 'failure to activate' or to bona fide repression. Moreover, this problem is complicated by the fact that it is impossible to observe repression in the absence of transcription. In previous *gal* experiments^{9–12}, catabolite repression was measured in medium containing both glucose and galactose because transcriptional activation occurs only in galactose medium. Thus, in this and other examples of catabolite repression, it is difficult to distinguish the effects of activation from those of repression.

In the present work, I fuse the 365-bp *gal10* segment to several *his3* segments containing the entire promoter region. In glucose-containing medium, the wild-type *his3* gene is transcribed at a basal level (1–2 messenger RNA molecules per cell) which is typical for yeast genes in general¹⁴. This basal level is not affected by the source of carbon in the medium¹³. Extensive deletion analysis has defined the *his3* promoter elements that are necessary and sufficient for this basal level of transcription: these include an upstream promoter element located 112–126 nucleotides upstream from the RNA initiation site (nucleotides –112 to –126) and a TATA element located between –32 and –52 (refs 15–17). In other words, the entire promoter region necessary for normal levels of *his3* transcription is contained within the 130-bp region immediately preceding the RNA coding sequences.

Thus, in this experimental scheme, catabolite repression is analysed under circumstances in which transcriptional activa-

Table 1 Structure and phenotypes of *his3* derivatives

DNA fragment	<i>his3</i> allele	Upstream endpoint	Downstream endpoint	IGP dehydratase activity	
				Glucose	Galactose
Sc3355	G12	<i>GAL</i>	–200	0.2	12
Sc3357	G13	<i>GAL</i>	–188	0.2	16
Sc3379	G14	<i>GAL</i>	–169	0.2	12
Sc3377	G15	<i>GAL</i>	–153	0.2	14
Sc3404	Δ71	–447	–200	1.0	0.9
Sc3405	Δ72	–447	–188	1.1	1.0
Sc3406	Δ73	–447	–169	1.0	1.1
Sc3387	Δ74	–447	–153	1.1	1.0
Sc3366	Δ75	–447	–131	1.0	1.0
Wild type	–	–	–	1.0	1.0

All DNA fragments were derived from Sc2812, and their corresponding *his3* allele numbers are prefaced by a G for *gal-his3* fusions or by a Δ for internal deletions. The upstream and downstream endpoints are listed (see Fig. 1 for details). Strains were grown in YP medium (1% yeast extract, 2% peptone) containing 2% of the carbon source to be tested (glucose or galactose). IGP dehydratase enzymes activities were determined by standard methods²¹ on chloroform-permeabilized cells from 15-ml cultures. Assays were performed in duplicate and values were normalized to the number of cells assayed. Activity levels are presented relative to that of wild-type cells (defined as 1.0); the error ~ ± 10%.

tion is achieved by a fully functional and heterologous promoter. In addition, by measuring *his3* levels in glucose medium, the repressing effects conferred by the *gal* segment can be separated from activating effects.

The DNA molecules used in these experiments were derived from YIp5-Sc2812, which contains the *ura3* selectable markers and a 6.1-kilobase (kb) *his3* DNA fragment (Fig. 1). The *gal* segment was fused to the *his3* gene at positions –200, –188, –169 and –153. As controls, the identical *his3* DNA segments were fused to position –447 instead of the *gal* DNA fragment; this position is located within the *per56* structural gene, and the adjacent sequences do not contain an upstream promoter element¹⁷. By selecting for *ura3*⁺ transformants, the *gal-his3* fusions and the control DNAs were integrated in single copy exactly at the normal *his3* chromosomal location (see Fig. 1 legend). The resulting strains were grown in appropriate medium and then assayed for *his3* expression either by assessing the level of IGP dehydratase (the *his3* gene product) (Table 1), or by determining *his3* RNA levels (Fig. 2).

When the strains were grown in glucose medium, the *gal-his3* fusions produced only 20% as much IGP dehydratase as both the control molecules and was the wild-type *his3* gene (Table 1). When the same strains were grown in galactose medium, *his3* was expressed at an extremely high level, which was indistinguishable from that observed with previous *gal-his3* fusions in which the *gal* element replaced the *his3* upstream element¹³. To prove that this 'glucose effect' occurs at the level of transcription, two of the *gal-his3* fusions and their respective control molecules were analysed for *his3* RNA production. As shown in Fig. 2, the *gal-his3* fusions (lanes 4, 5) produced undetectable quantities of RNA, whereas the control molecules (lanes 2, 3) produced similar levels to the wild-type strain (lane 6).

Table 2 Detailed phenotypes

<i>his3</i> allele	Upstream endpoint	Downstream endpoint	IGP dehydratase activity			
			Glucose	Glucose +AT	Raffinose	Raffinose +AT
G13	<i>GAL</i>	–188	0.2	0.9	1.1	3.2
G15	<i>GAL</i>	–153	0.2	0.7	1.0	2.9
Δ72	–447	–188	1.0	3.1	1.0	3.4
Δ74	–447	–153	1.1	3.0	1.1	3.1
Wild type	–	–	1.0	3.0	1.0	3.3

The *his3* allele numbers and the upstream and downstream endpoints are described in the text and in the legend to Table 1. Strains were grown in minimal medium containing 2% of the carbon source to be tested (glucose or raffinose). For conditions of amino-acid starvation, cells were collected 6–8 h after addition of aminotriazole (AT) to 10 mM. IGP dehydratase activities were determined as described in Table 1.

To prove that these effects are due specifically to catabolite repression of the intact *his3* promoter, two of the *gal-his3* fusions and their respective control molecules were analysed in more detail (Table 2). First, in the *gal-his3* fusions, the reduction of *his3* expression was not observed in raffinose medium, conditions in which catabolite repression does not occur⁵⁻⁹. This result indicates that the effects are caused specifically by catabolite repression. Second, in the control molecules, *his3* was expressed at the wild-type basal level in glucose, raffinose or galactose medium. This reaffirms that the *his3* sequences are fully competent for *his3* expression¹⁵⁻¹⁷, and that the glucose effects are due specifically to the 365-bp *gal* segment. Third, *his3* expression in all derivatives tested was induced during conditions of amino-acid starvation. This reaffirms previous observations that *his3* expression in *gal-his3* fusions depends specifically on *his3* promoter sequences¹³. This induced level is clearly subject to catabolite repression because in glucose medium the *gal-his3* fusions were expressed at only 20-30% of the level of expression of control molecules, whereas in raffinose medium, the fusions behaved similarly to the controls. Thus, catabolite repression of the *his3* gene is observed even when the *gal* regulatory site lies upstream of a functionally intact promoter region.

In many forms of regulation, RNA levels of a particular gene are higher in one circumstance than in another. However, it is impossible from such physiological phenomenology to determine whether this gene is subject to positive or negative control. For example, the term catabolite repression defines the lower levels of expression that are observed when glucose is the carbon source⁸. However, in *E. coli*, catabolite repression, despite its name, represents a positive control mechanism because the CAP regulatory protein activates transcription³. This activation requires cyclic AMP as a cofactor which binds to the CAP protein. Thus, as cells grown in glucose medium contain less intracellular cyclic AMP than cells grown in poorer carbon sources, catabolite repression is actually a consequence of the lack of a functional activator protein rather than the presence of a repressor protein.

The experiments reported here suggest strongly that catabolite repression in yeast indeed occurs by a negative control mechanism. The control region in the *gal* segment overrides the normal *his3* promoter elements and greatly reduces transcription. Such a result cannot be explained by the lack of a functional activator protein because if this were the case, normal levels of *his3* expression would be expected. Indeed, normal levels are observed in raffinose medium, conditions in which catabolite repression does not occur. Thus, catabolite repression is probably mediated by a repressor protein that recognizes sites in the *gal* segment and in analogous regions for other genes under similar control.

The experiments described here also demonstrate that catabolite repression and galactose activation are separable properties of the *gal* regulatory region. In particular, catabolite repression of *his3* expression is observed in the absence of galactose. This suggests strongly that these two regulatory phenomena are mediated, at least in part, by different DNA sequences in the *gal* segment. This notion is supported by deletion mutations of the *gal* segment which are defective in glucose repression yet are fully functional in terms of galactose activation¹². Note that the catabolite repression described here represents a 5-fold effect, whereas previous experiments (comparing cells grown in galactose with those grown in galactose plus glucose) indicated a 20-fold effect^{9,10}. This apparent difference probably results from inducer exclusion effects (cells prefer to transport glucose in preference to galactose)^{9,10} and effects on the binding of the *gal4* activator protein¹⁸. The experimental design used in the present work clearly distinguishes repression from these other effects, which both represent incomplete activation.

Prokaryotic repressor proteins inhibit transcriptional initiation by binding to sites that overlap the promoter region and thus interfere with functional RNA polymerase binding^{1,2}. Similarly, the purified simian virus 40 T-antigen binds to sites

with which transcription factors are known to interact¹⁹. In a somewhat different situation, the *E. coli* LexA protein represses galactose induction in yeast cells when its cognate operator site is located at various positions between the *gal* element and the TATA box²⁰. This effect is not observed when the *lexA* site is placed close to but upstream from the *gal* element.

In contrast, the catabolite repression site in the *gal* region exerts its effects even when located upstream of an intact promoter region. The *gal* site also appears to reduce expression when upstream of the *cyc1* promoter, although this is complicated by the fact that *cyc1* expression itself is subject to catabolite repression²⁵. In the derivatives examined here, the critical regulatory region is located ~100-150 bp upstream of the *his3* upstream promoter element^{12,17}. Because this is a rather large distance, and because the *his3* elements are intact, it seems probable that the mechanism of repression does not involve steric competition between the presumptive regulatory protein and the transcriptional apparatus.

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Nuclear extracts from globin-synthesizing cells enhance globin transcription *in vitro*

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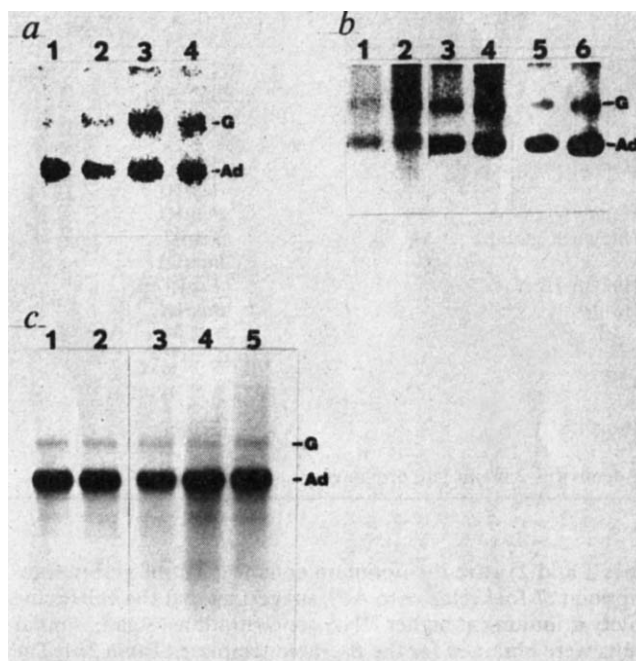
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In vitro transcription studies of cloned messenger RNA-coding genes have yielded considerable information regarding the sequence elements and protein factors involved in transcription initiation and RNA processing (reviewed in ref. 1). Fractionation of whole-cell², S-100 protein³ and nuclear extracts⁴ reveals the existence of both general class II⁵⁻⁹ and gene-specific^{8,10,11} transcription initiation factors. Because the soluble *in vitro* transcription systems prepared from cells in culture are largely nonspecific for the origin of the template DNA^{1,12}, they are highly suited to

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Fig. 1 Response of γ -globin (G) and adenovirus-2 major late promoter (Ad) transcription to the K562 nuclear extract. The products of the *in vitro* transcription reaction were electrophoresed in formaldehyde-containing gels. The upper band is the γ -globin transcript, 622 bases long, and the lower is the 442-base transcript from the Ad-2 major late promoter. The pyP4.0 γ -globin cloned DNA²¹ was digested with *Pst*I restriction endonuclease while the Ad-2 cloned DNA (the *Sma*II fragment containing the major late promoter; ref. 3) was digested with *Ava*II. **a**, Lane 1, control reaction in which 2 μ l of the KCl-containing buffer (used to wash the K562 nuclei) was mixed with 0.5 μ g DNA before transcription initiation. In lanes 2–4, the DNA was pre-mixed with 2 μ l of K562 extract containing 1×10^5 , 2.5×10^5 and 1×10^6 cell equivalents, respectively. The mass ratio of γ -globin to Ad-2 template was 5:1. **b**, Effect of template concentration. Transcripts from reactions containing 0.25 μ g (lanes 1, 2), 0.5 μ g (lanes 3, 4) and 1 μ g (lanes 5, 6) of total DNA. The mass ratio of globin to Ad-2 DNA was 5:1. The DNA was pre-mixed with 2 μ l of control buffer in lanes 1, 3 and 5, and with 2.5×10^5 cell equivalents of K562 extract in lanes 2, 4 and 6. **c**, Response of γ -globin and adenovirus major late promoter to a nuclear extract prepared from uninduced K562 cells. The control reaction (lane 1) was carried out by mixing 2 μ l of the KCl-containing buffer with 0.5 μ g DNA before transcription. In lanes 2–5, the DNA was pre-mixed with 1×10^5 , 2.5×10^5 , 5×10^5 and 1×10^6 cell equivalents, respectively. The mass ratio of the γ -globin to Ad-2 template was 5:1.

Methods. Whole-cell transcription extracts were prepared by the method of Manley *et al.*² from HeLa cells grown in suspension culture in Eagle's minimal essential medium containing 10% fetal calf serum. Transcription assays were performed in 50- μ l volumes. When more than one DNA template was used, the two DNAs were premixed and diluted to $0.2 \mu\text{g } \mu\text{l}^{-1}$ to minimize pipetting error, before adding aliquots to reaction tubes; 5 μ l of nucleoside triphosphates containing 5 mM ATP, CTP and UTP and 1 mM GTP were added together with 10 μ l of [α -³²P]GTP, water to raise the final volume to 50 μ l, and finally 30 μ l of the HeLa whole-cell extract. This extract contained 20 mM HEPES (pH 7.9), 0.1 mM EDTA, 12.5 mM MgCl₂, 100 mM KCl, 2 mM dithiothreitol and 17% glycerol. Incubations were for 1 h at 20–22 °C. Reactions were stopped by adding an equal volume of 8 M urea, 2% SDS, 40 mM EDTA and raising the temperature to 100 °C for 5 min. The mixtures were treated twice with phenol-chloroform, the aqueous phase was ethanol-precipitated and the pellets washed with 70% ethanol before denaturation and electrophoresis. The electrophoresis gel systems used were: (1) 7.5% polyacrylamide in 8.3 M urea; (2) 1.5% agarose after denaturation in glyoxal (described in ref. 22); and (3) 1.5% agarose containing formaldehyde (described in ref. 23). Human erythroleukaemia-like cells, K562, were grown in suspension in minimal essential media supplemented with sodium pyruvate, glutamine, 15% fetal bovine serum, non-essential amino acids and vitamins. Cells in log-phase growth were induced to synthesize haemoglobin by addition of haemin to a concentration of 25 μ M. The percentage of benzidine-positive staining rose to over 80% after exposure to haemin for 3–4 days. Cells were then washed three times in buffer containing 1 mM Tris, pH 7.6, 25 mM KCl, 0.9 mM MgCl₂, 0.14 mM spermidine, 1 mM sodium butyrate and 0.5 mM phenylmethylsulphonyl fluoride. Cells were resuspended in 35 ml of this buffer, allowed to swell on ice for 10 min, then Dounce-homogenized with a tight pestle. Approximately 10^8 nuclei were washed twice by centrifugation and resuspended in 0.5–1.0 ml of the same buffer, then incubated on ice for 15 min. After addition of KCl to bring the final concentration to 0.3 M, the nuclei were incubated for a further 30 min, with occasional stirring. The salt-washed nuclei were then pelleted and the supernatant or nuclear extract was drawn off, and aliquots were frozen at –70 °C. Activity was lost with multiple freezing and thawing.



searching for tissue-specific and gene-specific transcription regulatory factors. In the experiments reported here, we have added a nuclear extract prepared from human erythroleukaemia-like cells (K562, which can be induced to synthesize ϵ - and γ -globin mRNA and protein^{13,14}) to several deproteinized DNA templates, and monitored transcription levels in a HeLa cell-free transcription system². The K562 nuclear extract enhanced transcription of β -, ϵ - and γ -globin genes by as much as 30-fold compared with control non-globin templates. These results suggest the presence of a globin gene regulatory factor in erythroleukaemia cell nuclei.

To visualize and quantitate levels of particular RNA polymerase II transcripts, the template DNA was cleaved with a restriction endonuclease several hundred base pairs on the 3' side of the capping site³, generating a suitably sized 'run-off' transcript which could be visualized by electrophoresis and autoradiography. For all of the genes for which the capping sites have been determined, the run-off transcripts obtained in our cell-free assay corresponded to the expected lengths (Table 1). The effect of nuclear components from globin-synthesizing cells on the levels of transcription of various genes was determined using the cell-free assay. Nuclei were prepared from K562 cells which had been induced with haemin to synthesize globin¹⁴; at least 80% of the cells contained haemoglobin, as measured by benzidine staining. The nuclear extract was prepared by washing nuclei in a small volume of buffer containing KCl at 0.3 M (see Fig. 1 legend). Template DNAs were preincubated with the nuclear extract for 10 min at room temperature before addition of the HeLa whole-cell extract and ³²P-labelled and unlabelled nucleoside triphosphates. After incubation the RNA was purified and electrophoresed for analysis and quantitation of the effect of the K562 nuclear extract.

Figure 1a shows the effect of the K562 nuclear extract on

run-off transcription of a human γ -globin template (denoted G) and the adenovirus-2 major late promoter (referred to as Ad) when both DNAs were present in the same reaction. In the absence of the K562 extract (Fig. 1, lane 1), Ad transcripts predominated by a factor of greater than 10:1 even though the mass ratio of globin to Ad template DNA was 5:1. With increasing amounts of K562 extract, however, the level of globin transcription was markedly stimulated. Figure 1, lane 3, shows an 11-fold stimulation of globin transcription relative to Ad transcription, compared with the control reaction (lane 1). Different K562 nuclear extracts were found to stimulate γ -globin transcription 7–30-fold; this variability may be related to protein/DNA ratios, yields of the enhancing activity in different preparations, extent of haemin induction (see below), or to other factors. Preincubation of the nuclear extract and template was required for the stimulatory activity to be observed. Addition of the nuclear extract to DNA preincubated in the HeLa whole-cell extract did not result in stimulation of globin transcription; this may have been caused by competition between various DNA-binding proteins for the DNA template. The K562 extract had little or no effect on Ad major late transcription over the concentration range in which globin transcription was stimulated. On addition of further amounts of K562 nuclear extract, the level of globin transcription began to fall (Fig. 1, lane 4). In this range, the K562 extract did not significantly suppress Ad transcription, although greater amounts of extract in other experiments did reduce both Ad and globin transcription levels (data not shown). Similar results were obtained for the human β -globin gene (Table 2a). We next tested the effect of template concentration while holding the amount of K562 extract per reaction constant (at the amount found to be optimal in Fig. 1a). Figure 1b shows that the lowest template concentration

Table 1 *In vitro* templates

Gene	Restriction endonuclease cleavage site	Approximate length of run-off transcript (no. of residues)	Expected run-off length	Transcription efficiency (relative to γ -globin)
Human γ -globin	<i>Pst</i> I	620	622	-
	<i>Bam</i> HI	470	476	
Human ϵ -globin	<i>Bam</i> HI	480	480	1.0
Human β -globin	<i>Eco</i> RI	1,450	1,450	1.0
	<i>Bam</i> HI	480	480	1.0
Human <i>HLA</i>	<i>Eco</i> RI	500	Not determined	0.5
HSV <i>tk</i>	<i>Bam</i> HI	2,900	2,850	
		2,100	2,100	
		1,500	1,510	3.0
		900	940	
		570	570	
Hepatitis B virus	<i>Eco</i> RI	330	334	0.8
		900	875	
Adenovirus-2 major late promoter	<i>Ava</i> II	450	442	50.0

(lanes 1 and 2) gave the optimum enhancement of globin transcription (17-fold relative to Ad), suggesting that the enhancing activity is limiting at higher DNA concentrations. Again, similar results were obtained for the β -globin template (Table 2b). The implications of β -globin transcription are discussed below.

We find that the stimulatory activity is not dialysable; it is sensitive to heating at 65 °C for 10 min; and the activity is eluted from an anion-exchange resin, DEAE-cellulose (DE52), at a KCl concentration of 0.15 M. Therefore we conclude that the transcriptional enhancing activity is due to a protein component(s). We find that induction of the K562 cells with haemin is necessary for activity. Extracts prepared from uninduced cells, in which the constitutive level of benzidine positive-staining cells was less than 4%, had no enhancing effect on globin gene transcription (Fig. 1c). A 0.3 M KCl wash of chromatin isolated from hypotonically lysed HeLa cell nuclei had no enhancing activity (Fig. 2a). A similar extract prepared from human hepatic carcinoma cells also had no effect on globin transcription; further, a low-ionic strength wash of induced K562 cell nuclei showed no enhancing activity (Fig. 2b).

The observation that at a fixed amount of K562 extract maximum enhancement is found at the lowest template concentrations, suggests that the enhancing factor does not act catalytically and probably does not cycle between templates. To test for the formation of a stable complex between the enhancing factor and the DNA, an order-of-addition experiment was carried out. The γ -globin DNA was digested with two different

restriction endonucleases so that two transcripts of different length would result from a mixed template reaction. One of the templates was preincubated with the K562 extract before the addition of the second template. After a second incubation period, transcription was initiated in the standard fashion by adding nucleoside triphosphates and the HeLa cell-free extract. In the control reaction (Fig. 2c, lane 1), the first template (cleaved with *Pst*I, which gave rise to the longer transcript) was mixed with an equivalent amount of buffer while in the reactions of lanes 2–4 the first template was mixed with increasing amounts of K562 nuclear extract. At the lower concentration of added extract (5×10^4 cell equivalents, lane 2) transcription from the first template added (*Pst*I) was markedly enhanced relative to that from the second template (*Bam*HI). With higher concentrations of extract, transcription was stimulated from both templates. Even with higher extract concentration the first template was preferentially transcribed over the second. To test that the response to the extract was independent of the DNA sequences between the two 3' restriction sites, the order of addition of the templates was reversed. Identically, transcription from the first template (*Bam*HI-cleaved) was enhanced relative to that from the second template (*Pst*I-cleaved, data not shown). The level of enhancement of transcription from the first template is highly dependent on the amounts of both DNA and extract used; however, enhancement of transcription from the first template is independent of the amount of the second template. These data indicate that the K562 extract contains a non-cycling DNA-

Fig. 2 a, Effect of a nuclear extract prepared from HeLa cells on globin transcription. Products of transcription from the γ -globin template were electrophoresed in a 7.5% polyacrylamide gel containing 8.3 M urea. In the reaction corresponding to lane 1, 0.5 μ g of DNA was mixed with a control buffer, before transcription initiation, and in the other reaction (lane 2), the DNA was mixed with 2.5×10^5 cell equivalents of nuclear extract prepared from HeLa cells in the same manner as the nuclear extract from K562 cells. M denotes length markers. **b**, Effects of KCl concentration on elution of transcription-enhancing activity. Products of transcription from the γ -globin template were electrophoresed in a 7.5% polyacrylamide gel containing 8.3 M urea. The control reaction (lane 1) contained 2 μ l of control buffer (transcription buffer). The reaction corresponding to lane 2 contained 2×10^5 cell equivalents of K562 extract prepared as described above, using 0.3 M KCl, and dialysed against the control buffer. In the third reaction (lane 3), the DNA was mixed with a K562 extract prepared by washing nuclei in 50 mM KCl followed by dialysis against the control buffer. The level of transcription shown in lane 2 is 10-fold that shown in lane 1 or 3. **c**, Products of *in vitro* transcription from a mixture of two γ -globin templates, one digested with *Pst*I and the other with *Bam*HI. The upper band corresponds to the 622-base run-off transcript from the larger template (*Pst*I) and the lower band corresponds to the 474-base transcript from the shorter template (*Bam*HI). The control reaction (lane 1) involved pre-mixing 0.5 μ g of the larger template with control buffer, and in lanes 2–4 with 5×10^4 , 2.5×10^5 and 5×10^5 cell equivalents of K562 nuclear extract, respectively. The mixtures were incubated for 10 min at 22 °C before the addition of 0.5 μ g of the *Bam*HI-cleaved template. A second incubation of 10 min at 22 °C preceded the initiation of transcription as described. The ratio of transcription from the longer template to that from the shorter template is shown below each lane (P/B). The transcripts were electrophoresed in 0.5% agarose after denaturation with glyoxal.

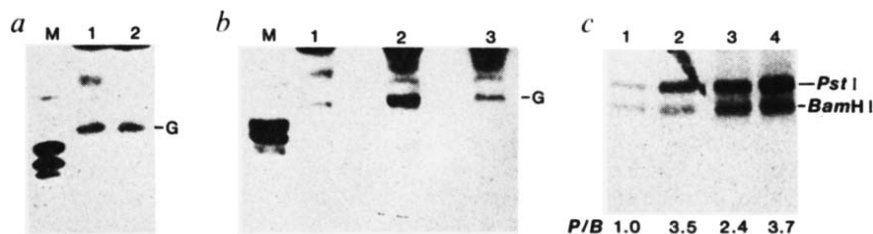
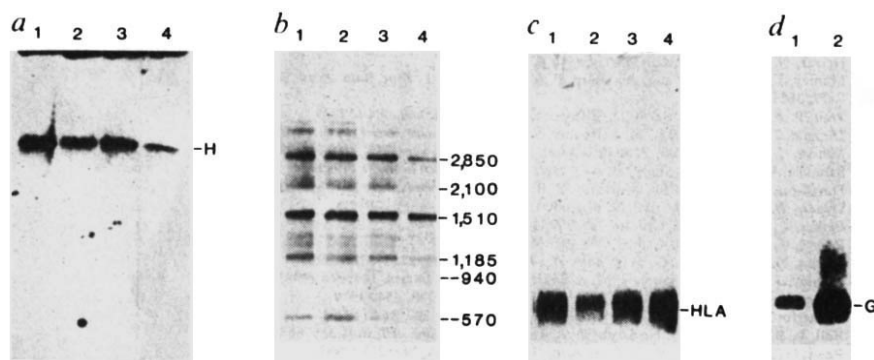


Fig. 3 Effect of the K562 nuclear extract on transcription of other polymerase II genes. **a**, Products of transcription from 0.5 μ g of hepatitis B virus DNA (digested with *Eco*RI; ref. 15) were electrophoresed in a 7.5% polyacrylamide gel containing 8.3 M urea. The major transcript is approximately 320 bases long. Lane 1 shows the product of the control reaction, while the reactions in lanes 2–4 contain 1×10^5 , 2.5×10^5 and 5×10^5 cell equivalents of K562 nuclear extract, respectively. **b**, Products of transcription from 0.5 μ g of the herpes simplex virus *tk* gene were electrophoresed in 1.5% agarose after denaturation in glyoxal. The *tk* cloned DNA¹⁶ was cleaved with *Bam*HI restriction endonuclease. Lane 1 shows the transcription products of the control reaction, while the reactions in lanes 2–4 contained 1×10^5 , 3×10^5 and 6×10^5 cell equivalents of K562 nuclear extract, respectively. **c**, Products of transcription from 0.5 μ g of the human class I *HLA* gene were analysed in a 1.5% agarose gel after glyoxal denaturation. The product of the control reaction is shown in lane 1; lanes 2–4 show the products of reactions containing 5×10^4 , 2×10^5 and 8×10^5 cell equivalents of K562 nuclear extract, respectively. **d**, Products of transcription from 0.5 μ g of the human ϵ -globin²¹ gene were analysed in a 1.5% agarose gel after denaturation with glyoxal. The ϵ -globin cloned DNA was cleaved with *Bam*HI. The product of the control reaction is shown in lane 1; lane 2, the product of a reaction containing 2.5×10^5 cell equivalents of the K562 nuclear extract.



binding activity which is responsible for stimulation of globin transcription.

To determine whether transcription enhancement of γ - and β -globin genes was specific beyond the comparison with the Ad-2 major late promoter, other globin and non-globin polymerase II genes were tested. Figure 3a shows the major transcript obtained from the hepatitis B virus (HBV) surface-antigen gene in the HeLa cell-free assay¹⁵. At protein/DNA ratios spanning those in which the K562 nuclear extract enhanced globin transcription, no positive effect on HBV transcription was observed (Fig. 3a). Another polymerase II promoter of viral origin was tested for its response to the K562 nuclear extract. The complex pattern of transcripts obtained from the thymidine kinase gene (*tk*) of herpes simplex virus (HSV) was comparable to that described previously¹⁶ (Fig. 3b). Similar to the result for HBV, the K562 extract has no effect on the levels of any of these transcripts. In addition to polymerase II promoters of viral origin, a human class I histocompatibility locus antigen gene (*HLA*) was transcribed in the cell-free system with an efficiency of ~50% that of γ -globin; this gene gave rise to a single diffuse transcript, possibly indicating an unusual secondary structure.

Table 2 Transcription enhancement of β -globin as a function of amount of K562 nuclear extract (a) and template concentration (b)

a		Transcripts per gene per h*		Enhancement
K562 extract (cell equivalents)				
0		0.004 ± 0.002		—
5×10^4		0.008		2×
1×10^5		0.022		6×
2×10^5		0.068		17×
5×10^5		0.070		18×
1×10^6		0.058		15×

b		Transcripts per gene per h (± 0.002)		Enhancement
DNA (μ g)		— extract	+ extract	
0.25		0.006	0.024	4×
0.5		0.006	0.164	30×
1.0		0.092	0.270	6×
2.0		1.368	1.356	1×
4.0		0.616	0.622	1×
8.0		0.066	0.058	1×

In a, 0.3 μ g β -globin template DNA was used. In b, 2.5×10^5 cell equivalents of K562 extract were used.

* Calculated from the specific activity of 32 P-GTP, the number of G residues in the run-off transcript, number of genes in the reaction, and the d.p.m. incorporated (corrected for counting efficiency).

Over a 16-fold range of DNA/K562 nuclear extract ratios, no enhancement of transcription was observed (Fig. 3c).

Of the polymerase II genes described so far, only the γ -globin and β -globin templates responded positively to the enhancing activity of the K562 nuclear extract. In addition to these two globin genes, the third human β -like gene, ϵ -globin (embryonic), was also tested for its response in the transcription assay; it is also synthesized in K562 cells after induction^{14,17}. Similarly, there was a 20-fold enhancement of ϵ -globin transcription in the cell-free assay after preincubation with the nuclear extract (Fig. 3d). We have not yet tested the effect of the K562 extract on human α -globin transcription. It is curious that the K562 extract stimulated β -globin transcription (Table 2) as K562 cells express only the γ and ϵ genes and not the β gene¹⁷. The presence of a β -globin gene family transcription factor may be a necessary but not a sufficient condition for transcription *in vivo*. In the *in vitro* experiment, the effect of the developmental history of the gene is lost and the roles of epigenetic factors such as chromatin structure^{18,19} or specific chromatin-bound non-histone proteins are not addressed.²⁰

Although the data presented above support the existence of a globin gene-specific positive transcription factor, the effect of the K562 nuclear extract on RNA stability must also be considered. To test for preferential stabilization of globin RNA, we synthesized a mixture of γ -globin and Ad major-late run-off transcripts in the HeLa system. RNA was purified and then resuspended either with the K562 nuclear extract or the equivalent buffer. A second incubation (1 h) in the HeLa extract preceded final RNA purification and gel analysis. The ratio of globin to Ad transcripts was identical for the experimental and control reactions, indicating that the K562 extract does not preferentially stabilize globin transcripts. We conclude that the K562 nuclear extract contains a factor which markedly stimulates transcription from the β family of human globin genes. Assuming that only one molecule of this factor is bound per utilized gene, we estimate that the abundance of this factor is 10^3 – 10^4 copies per K562 cell. This estimate is based on the number of cell equivalents at optimal enhancement (5×10^5) and the number of active gene copies in the reaction (1–10% of 5×10^{10} genes; ref. 12). Purification of this nuclear activity and determination of its site of interaction with the β -globin genes must now be attempted.

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Identification of multiple metal regulatory elements in mouse metallothionein-I promoter by assaying synthetic sequences

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Metallothionein genes are transcriptionally regulated by a number of inducers including heavy metals. Previous mutational analyses of the mouse metallothionein-I gene (*mMTI*) promoter have delineated a heavy-metal regulatory region between -60 and -42 relative to the transcription start site^{1,2}. A synthetic copy of a 12-base-pair (bp) conserved sequence located within this region was subsequently shown to confer heavy-metal regulation on a heterologous gene^{1,3}. However, specific disruption of this metal regulatory element (MRE) within a wild-type *mMTI* promoter reduced but did not eliminate the heavy-metal response. The additional metal regulatory activity was localized to an upstream region containing four sequences homologous to the identified MRE¹. Similar sequences were also found in multiple copies in metallothionein genes from other species. Here we test synthetic copies of all five *mMTI* MRE homologues for metal regulatory activity. At least four of these sequences are able to confer heavy-metal regulation on a heterologous promoter.

Figure 1a shows the five MRE homologues of the *mMTI* promoter⁴ and similar sequences found in the promoters of five other metallothionein genes from man^{5,6}, mouse⁷, sheep⁸, and *Drosophila* (J. Mercer and G. Maroni, personal communications). A consensus derived from these sequences is presented at the bottom of Fig. 1a. A synthetic copy of a sequence containing most of the promoter-proximal *mMTI* MRE homologue (MRE-a', Fig. 1b) was previously shown to confer heavy-metal inducibility on a herpes simplex virus (HSV) thymidine kinase gene (*tk*) when inserted into the promoter region. The response to heavy metal was relatively independent of the position or orientation of the MRE, but required at least two copies of the MRE sequence³. The function of the remaining sequences in Fig. 1 have not been tested directly, but as indicated above, previous results suggest that at least one other MRE exists within the *mMTI* promoter. In addition, Karin *et al.*⁸ have shown that two regions of the human metallothionein-II_A (*hMTIIA*) promoter independently respond to heavy metals. One of these regions contains the *hMTIIA* MRE homologues d and e and

mMT-I	a	-54	CTTTGGCGCGGCACT	-40	ori
	b	-56	GTTTGACCCAGCAG	-70	r
	c	-132	AAGTGGCTCGGCTC	-118	n
	d	-150	CTCTGCACTCCGCC	-136	n
	e	-175	CTGTGCACTGGCG	-161	n
hMT-II _A	a	-11	CGCTGCACTCCACCA	+4	n
	b	-57	TTTTGCATCGTCCC	-43	n
	c	-74	GGCTGCACAGCCCC	-88	r
	d	-139	CAGTGGCGGGCGCG	-125	n
	e	-153	GGGTGGCGGGCGCC	-139	n
	f	-156	CTCTGCACAGGGCC	-170	r
	g	-245	CGCTGCACACGACC	-231	n
	h	-305	CTGTGCACACGGCG	-291	n
hMT-I _A	a	-58	CTTTGCGTCCGGCC	-44	n
	b	-60	GGCTGGCGCGGCC	-74	r
	c	-150	TGCTGGCGAAGGCC	-136	n
	d	-168	CTGTGGCGCTTGCT	-154	n
	e	-420	GGCTGCACCTAGACT	-406	n
	f	-460	CTCTGCATACGCGC	-446	n
	g	-499	CTCTGCACCAAACT	-485	n
mMT-II	a	-58	TTTGGCGTCCGACC	-44	n
	b	-80	CGATGCAGGGCGCC	-94	r
	c	-99	GGCTGCACAGCCCT	-113	r
	d	-135	GGCAGCAACGCCCC	-149	r
	e	-153	GGCTGGGCAAGGCC	-167	r
	f	-207	GGCTGCACAGCCCT	-221	r
	g	-297	CTTTGGCGTCAGCT	-283	n
sMT-I	a	-54	CTCTGGCGGGCGCC	-40	n
	b	-66	GGCTGCACCGAGCT	-80	r
	c	-84	CGCTGCACCGGGTC	-98	r
	d	-132	TCTTGGGCTGGGCG	-118	n
	e	-165	GGGTGGCGAGGAGC	-151	n
	f	-435	CTCTGCACAGCAC	-421	n
dMT	a	-12	AGATGCTCTCGGTT	-26	r
	b	-47	CTTTACACAGGGTC	-61	r
	c	-79	TTTGGCAACGCGG	-93	r
	d	-107	ATTGGAGCGGGCG	-93	n
	e	-128	TTCTGCACAGCTCT	-114	n
CONSENSUS			CTNTGCRNCGGGCC 55-00000-976775		
b	MRE-a'	a	<u>GATCCCTTTGGCGCGCA</u>		
		b	<u>GGAAACGCGGCGTCTAG</u>		
	MRE-a	a	<u>GATCCCTTTGGCGCGGACTA</u>		
		b	<u>GGAAACGCGGCGCTGATCTAG</u>		
	MRE-b	a	<u>GATCCGTTTGACCCAGCAGA</u>		
		b	<u>CGAAACGTGGGCTGCTCTAG</u>		
	MRE-c	a	<u>GATCCAAGTGGCTCGGCTCA</u>		
		b	<u>GTTTCAGCGGAGCCGAGTCTAG</u>		
	MRE-d	a	<u>GATCCCTCTGCACTCCGCCCA</u>		
		b	<u>GGAGACGTGAGGCGGGCTAG</u>		
MRE-d'	a	<u>GATCCCTCTGCACTCCGCCCGA</u>			
	b	<u>GGAGACGTGAGGCGGGCTCTAG</u>			
MRE-e	a	<u>GATCCCTGTGCACACTGGCGA</u>			
	b	<u>GGACACGTGTGACCGGCTCTAG</u>			
MRE-h	a	<u>GATCACCTCGTCCGGGCTCTA</u>			
	b	<u>GTGACGAGGCGGAGGACTAG</u>			

Fig. 1 *a*, MRE homologues of metallothionein gene promoters from several species. Lowercase letters preceding the *MT* gene listings indicate the species of origin: h, human^{5,6}; m, mouse^{4,7}; s, sheep; d, *Drosophila* (J. Mercer and G. Maroni, personal communication). Sequences are written 5' to 3'; the homologues within each promoter are listed in order beginning closest to the cap site. The positions of the end nucleotides of a given sequence within the promoter are given on either side of the sequence. The orientation (ori) of the sequences within each promoter is indicated to the right: n, normal; r, reversed. The *mMTI* and *hMTIIA* homologues were initially identified in a cross-comparison using the homology matrix described in ref. 17. The derived consensus sequence is also given, with the consensus nucleotide frequency at each position indicated below: 5 = 50%, 6 = 60%, ... 9 = 90%, 0 = 100%. *b*, Both strands of the synthetic MREs corresponding to homologues a-e (MRE-a to MRE-e) of the *mMTI* promoter are shown with the top strand listed 5' to 3'. Also shown is the sequence of the previously tested MRE-a' (referred to previously^{1,3} as MRE-a) and the sequences of MRE-d' and MRE-h (see text). MRE-h is displaced 6 nucleotides to the right to indicate our alignment of this sequence relative to the MRE consensus. All elements were synthesized with a *Bam*HI site on the left and a *Bgl*II site on the right (underlined). The single *Bam*HI site regenerated on insertion facilitated the analysis of insert orientation.

most of homologue f, while the other contains homologue a and part of homologue b (Fig. 1a).

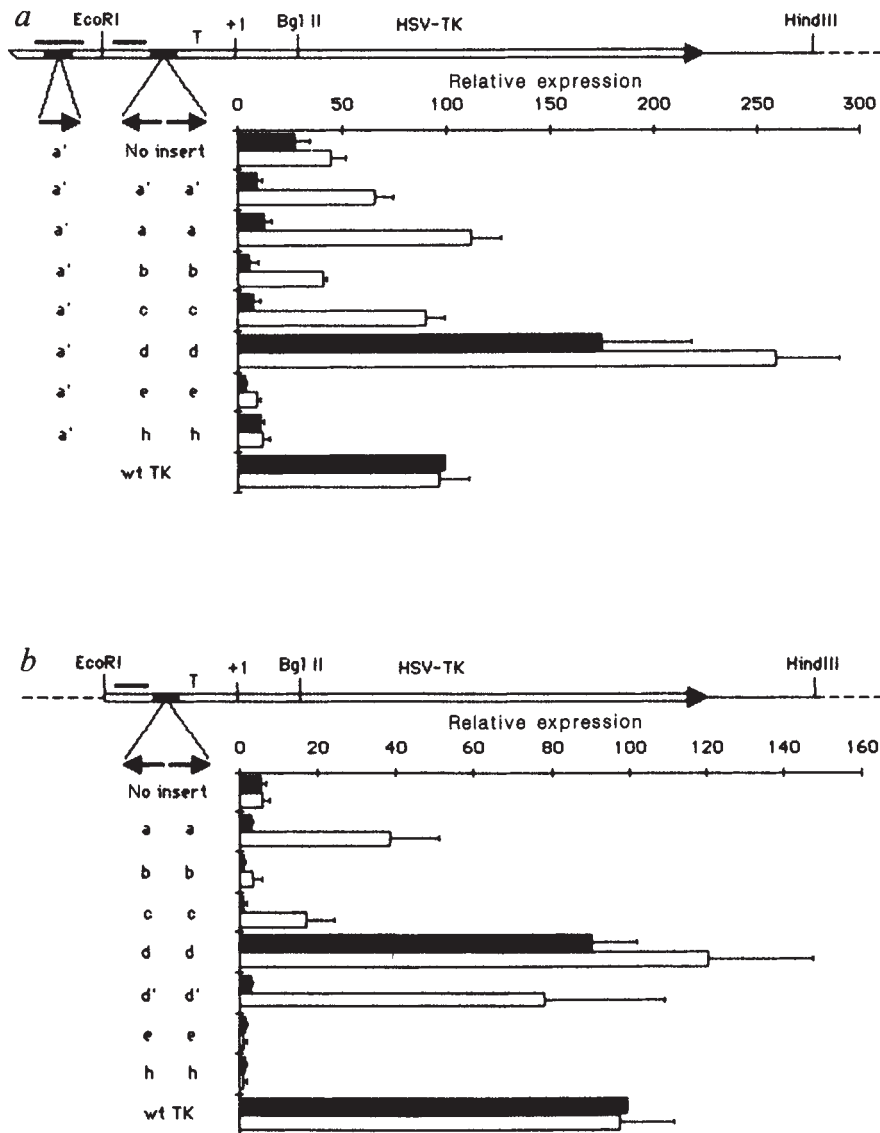
To verify the metal regulatory function of individual sequences, all five MRE homologues from the *mMTI* promoter, including an extended version of MRE-a' (Fig. 1b), were synthesized and tested for regulatory activity when inserted into the promoter of the HSV *tk* gene. Because *MT* promoters contain multiple

Fig. 2 a, Analysis of MRE function in a disrupted *tk* promoter. A diagram of the HSV *tk* double linker-scanning mutant into which the MRE sequences were inserted is shown at the top. The gene was formed by recombining LS-105/-95 with LS -42/-32 at the *EcoRI* site within the *tk* promoter¹⁰. Solid bars indicate the positions of the two *Bam*HI linkers disrupting the wild-type promoter. Open bars represent *tk* sequence with the direction of transcription indicated by the arrow. Lines above the *tk* promoter indicate the normal positions of the proximal and distal G+C-rich *tk* promoter elements; the latter is disrupted by a *Bam*HI linker. The position of the TATA box is indicated by 'T' and the normal start site for transcription as indicated by '+1'. All constructs are maintained in a pBR322-derived vector (dashed line) in which the *Pvu*II/*Hind*III *tk* gene fragment is inserted between the corresponding sites in the plasmid. The identities and orientations of the MRE sequences inserted into the *tk* promoter are shown below the promoter and to the left. Synthetic DNA insertions were accomplished using the procedures described³. Relative expression measured by transient transfection of these constructs into BHK cells (see below) is shown to the right. Open bars represent Zn-induced activities; solid bars are uninduced activities. Each value represents the average of three or more independent measurements with the standard deviations indicated. The results obtained with LS-119/-109, a *tk* mutant with wild-type expression levels¹⁸, are also shown for comparison (wt *tk*). **b**, Analysis of MRE function in a truncated *tk* promoter. The diagram of the gene is the same as in **a** except that the *tk* promoter sequences upstream of the *EcoRI* site (including MRE-a') are no longer present. The relative expression exhibited by this series of constructs is presented in the same format as in **a**.

Methods. Transfection assays were performed essentially as described elsewhere³, with some modifications. Duplicate cultures of subconfluent BHK cells in 6-cm plates were treated for 6-8 h with 0.4 ml of a 1-ml calcium phosphate precipitate containing 5 µg MRE test plasmid (4.0 kilobases), 5 µg pMT-βgal reference plasmid¹ (14.5 kilobases) and 10 µg herring sperm DNA in the presence of 0.1 mM chloroquine. The cells were then rinsed with saline and incubated overnight at 37 °C in normal media (Dulbecco's modified Eagle's medium + 10% fetal calf serum). 24 h after transfection, the media were changed and 120 µM zinc sulphate was added to one of each pair of cultures. After 18-22 h, the cultures were collected by scraping the cells into 0.2 ml homogenization buffer, sonicating, centrifuging for 10 min in a microfuge and freezing the supernatant at -70 °C. Thymidine kinase and β-galactosidase activities were determined from a 27-µl assay mixture containing 15 µl assay buffer and 12 µl cell extract (diluted when necessary with homogenization buffer) as described elsewhere³. β-Galactosidase values provided an internal control for variability in the transfection procedure. The values for relative expression of the test promoters have been normalized relative to the expression of the MT-βgal reference gene. This normalization step compares uninduced test promoters with the uninduced MT promoter, and zinc-induced test promoters with the induced MT promoter. The two sets of data are related using the mean induction of the MT promoter (17.8-fold). Use of the MT-βgal as an internal control reduces the standard deviations of the values obtained for relative expression.

MRE-like sequences, and because we have shown a requirement for at least two MREs to obtain significant induction when assayed in baby hamster kidney (BHK) cells³, we initially tested a series of constructions in which two copies of the MRE homologue to be tested were inserted as an inverted repeat into the proximal *Bam*HI site of a double linker-scanning mutant of the *tk* gene (see Fig. 2a). In addition, the previously defined MRE (MRE-a') was inserted into the distal *Bam*HI site. We reasoned that the presence of three MRE sequences, one of which was known to be functional, would allow partially responsive MREs to be more easily identified.

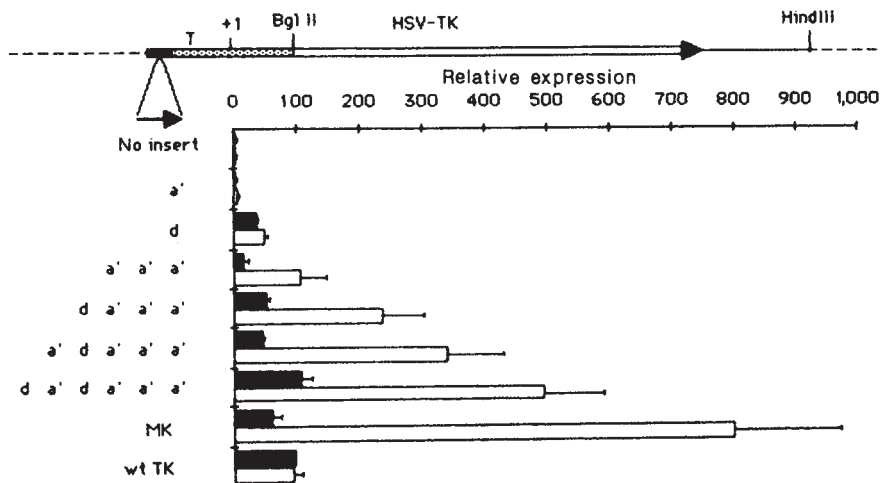
Gene expression in the presence and absence of heavy metal was evaluated after transient expression in BHK cells using the procedures outlined previously³ and described briefly in Fig. 2 legend. Because most transcripts initiate at the normal cap site in this system (ref. 9 and our unpublished results), the messenger



RNAs produced from the different transfected genes will be identical, and so the relative transcriptional rates from the different promoters should be accurately reflected in the relative accumulation of TK activity. The TK activity assay is very sensitive, allowing accurate quantitation of activity from even the weakest promoters. Each construct was tested in at least three separate experiments, and a reference gene was co-transfected with each test plasmid as an internal control (see Fig. 2 legend for details).

Figure 2a shows the relative expression of these constructs in the presence and absence of 120 µM zinc (Zn). Since the distal G+C-rich promoter element of the *tk* gene is disrupted by the distal *Bam*HI linker and MRE-a' inserts in these constructs, expression in the absence of heavy metal was low relative to that of a wild-type *tk* gene. The insertion of two additional MREs into the proximal *Bam*HI site generally caused further

Fig. 3 Modelling the wild-type *mMTI* promoter response with synthetic MRE sequences. A diagram of a 5' deletion of the *MK* fusion gene is shown at the top. The construction of this deletion mutant is described in ref. 1. Open bar, *tk* gene sequence; stippled bar, *mMTI* sequence; dashed lines, pBR322 vector sequence. The position of the TATA box is indicated by 'T' and the normal start site for transcription by '+1'. Various MRE sequences inserted into the *Bam*HI site (solid bar) defining the end point of the deletion are shown below the gene and to the left. All inserts were placed in tandem and in the same orientation in which they appear in the wild-type *mMTI* promoter. Relative expression is presented as in Fig. 2. Corresponding values for the undeleted gene (*MK*) and a wild-type *tk* gene (wt. *tk*) are also indicated.



reductions in uninduced expression. These results are consistent with the observed effects of spacing on *tk* promoter element function¹⁰. As observed previously³, three copies of MRE-a' in this configuration allowed a five to sixfold increase in TK activity after Zn treatment. However, replacing the downstream inverted repeat with an extended version of this sequence (MRE-a) produced an eightfold induction. This suggests that the four additional nucleotides at the 3' end of this sequence contribute to MRE activity, although the increased size of the insert may also have affected induction indirectly by changing the spacing between MREs or their positions relative to other promoter sequences. Both MRE-b and MRE-c also exhibited clearly significant metal regulatory activities. In contrast, the construct containing MRE-e responded poorly to Zn treatment. Hence, it is possible that some of the MRE homologues shown in Fig. 1a have diverged into inactive forms or only resemble functional MREs. Alternatively, the MRE-e sequence may function in a wild-type *mMTI* promoter but not in our rearranged *tk* promoter, where the number and/or placement of promoter elements could be suboptimal. Another possibility is that the wild-type *mMTI* promoter on the co-transfected pMT- β gal reference plasmid competes for limiting quantities of a diffusible regulatory factor, such that a weak regulatory element would appear non-functional. Competition between *MT* promoters has been observed by Seguin *et al.*¹¹; however, they noted a requirement for high concentrations of competitor DNA and used a Simian virus 40 (SV40) replicating vector system. We have not observed improved regulation of weakly inducing promoters transfected in the absence of the *MT*- β -galactosidase (*MT*- β gal) gene, and consider it unlikely that diffusible regulatory factors are limiting in our assay.

In addition to the *mMTI* MRE homologues, we tested a synthetic version of the downstream *hMTIIA* metal regulatory sequence proposed by Karin *et al.*⁸ (MRE-h, Fig. 1b). This sequence overlaps only the 3' end of *hMTIIA* homologue b shown in Fig. 1a. The sequence appeared to be unresponsive to heavy metals (Fig. 2). We conclude that the extent of homology of this sequence with our consensus is insufficient for regulatory activity.

Surprisingly, two copies of MRE-d allowed a 20-fold increase in TK activity in the absence of heavy metal (Fig. 2a). A sequence at the 3' end of this element (ACTCCGCCCA) shows a perfect match with sequences in the 21-bp repeats of SV40 and a 9 out of 10 bp match with a sequence in the distal G+C-rich *tk* promoter element (ACCCCGCCCA). Both the SV40 and *tk* sequences are involved in the binding of the transcription factor Sp1 (ref. 12 and K. Jones and R. Tjian, personal communication). Furthermore, Sp1 has recently been shown to 'footprint' several sequences within the *hMTIIA* gene (W. Lee and M. Karin, personal communication). Hence, it seems possible that the

metal-independent activity of MRE-d results from its interaction with Sp1 or its equivalent in BHK cells.

Although MRE-d seems to respond slightly to the presence of heavy metals, the 1.4-fold induction observed is less than the barely significant 1.5-fold induction of the control construction containing only MRE-a' in the upstream position (Fig. 2a). To test whether MRE-d and the other MREs were capable of sustaining an independent response to metals in the absence of MRE-a', we also tested a series of genes lacking the distal MRE. These were made from the previous series by deleting the promoter sequences upstream of the *Eco*RI site in the *tk* promoter. As shown in Fig. 2b, the loss of the upstream promoter sequences reduced both induced and uninduced levels of expression; however, the induction ratios observed with MRE-a, MRE-b and MRE-c (about 13-fold, 3-fold and 10-fold, respectively) were similar to those obtained with the previous series of constructions. As expected, the gene lacking any MRE insert was unresponsive to heavy metal. Similarly, genes containing MRE-e or MRE-h inserts showed no induction. Although MRE-d again appeared to have slight regulatory activity on its own, the observed induction was equivalent to the experimental error.

The A residue representing the 3' end of the Sp1 binding site homology of MRE-d is actually derived from the *Bgl*II site engineered onto the end of this synthetic sequence; the wild-type *mMTI* promoter sequence contains a G residue at this position. It was therefore unclear whether the wild-type sequence also acts as a metal-independent promoter element. To answer this question, we tested a modified version of MRE-d in which a G residue was inserted between the last A of the Sp1 homology and the preceding C (MRE-d', Fig. 1b). This change extends the homology with the corresponding *mMTI* promoter sequence by two additional nucleotides. When present as an inverted repeat in the deleted *tk* promoter construct, the modified sequence exhibited little or no basal activity, but allowed a 20-fold induction (Fig. 2b). Hence, a single base insertion converted the apparently metal-independent promoter activity of MRE-d into a metal-dependent promoter activity. This effect is correlated with the disruption of the Sp1 binding site homology. Since this change does not affect sequence homologous to other metal-responsive elements, it seems likely that the synthetic MRE-d contains two transcription factor binding sites. Competition between an Sp1-like protein and a metal-responsive transcription factor for overlapping binding sites may be responsible for the poor induction observed with constructs containing MRE-d.

The availability of synthetic sequences providing inducible and basal promoter element functions prompted us to attempt to model the expression and regulation obtained with a wild-type promoter. For these experiments, we used a *mMTI*/*tk* fusion gene (*MK*) in which *mMTI* promoter sequences were joined

to *tk* coding sequences at a convenient *Bgl*II site located within the untranslated leader region of both genes. As demonstrated previously³, this construct was highly inducible whereas a 5' deletion mutant extending to sequence position -42 (5' Δ -42) gave a low, uninducible level of TK activity (Fig. 3). The insertion of three copies of MRE-a' into the *Bam*HI site defining the end point of the deletion reconstituted a five-fold response to heavy metal. The addition of one MRE-d sequence immediately upstream of these three elements approximately doubled the expression of *tk* activity in both the presence and absence of Zn. Although MRE-d may have some capacity to respond to heavy metals, this relatively large increase in the induced level of expression could also result from the demonstrated ability of MRE-a' to interact with basal promoter elements³. The presence of an additional MRE-a' upstream of MRE-d in the latter construction caused only a slight increase in inducibility. However, the subsequent insertion of another copy of MRE-d increased both induced and uninduced expression, allowing an induced level of *tk* activity roughly equivalent to 70% of that obtained with a wild-type promoter. Although the induction observed with these model genes was slightly less than that observed with a wild-type promoter, it appears that both basal and metal-induced expression from a wild-type promoter may be accounted for by the action of two classes of promoter elements interacting with the *mMTI* 'TATA box' region. These results also demonstrate that promoter strength may be directly modulated by changing the copy number of individual promoter elements.

We have used synthetic oligonucleotides to test the function of specific MRE sequences found within the *mMTI* promoter. We have shown that four of the five MREs we recognized previously are individually able to confer metal responsiveness to the *tk* promoter when present as dimers. A comparison of the metal-responsive homologues suggests that the heptanucleotide TGCRCYC (R = purine, Y = pyrimidine) constitutes the core of a metal-dependent promoter element, which may be the recognition sequence for a metal-dependent transcription factor. This sequence closely resembles the nearly invariant consensus sequence TGCRCNC derived in Fig. 1a. It is noteworthy that this conserved minimal MRE sequence is small, and thus would be expected to occur frequently in the genome just by chance. The fact that relatively few genes are inducible by heavy metals can be attributed to the observed requirement for clustering of two or more such sequences within a promoter region in order to obtain a response to heavy metals³. The theme of multiple copies of relatively small functional sequences appears to be common among eukaryotic promoters and enhancers⁷⁻¹⁵. Although instances in which enhancer function is generated by duplication of a sequence present only once in the wild-type lead one to question the biological relevance of the single copy¹⁶, the fact that multiple related MRE sequences are found within *MT* promoters justifies our approach of examining the activity of duplicated elements.

We have also shown that the metal-independent promoter activity exhibited by one of our synthetic sequences is correlated with the presence of a strong Sp1 binding site homology within this sequence. Although the activity of this sequence apparently resulted from the chance juxtaposition of a partial Sp1 binding site homology found within the *mMTI* promoter and a synthetic *Bgl*II site, this finding may still be relevant to *mMTI* promoter function. One possibility is that metal-dependent regulatory factors enhance the binding or activity of other transcription factors such as Sp1, a suggestion strengthened by our previous observations of cooperative interactions between MREs and *tk* promoter elements³, and by the recent discovery of multiple Sp1 binding sites in the *hMTIA* gene promoter (W. Lee and M. Karin, personal communication). In addition to the observation that MREs and Sp1 binding sites coexist within *MT* gene promoters, we also note that many of the MRE homologues in Fig. 1a contain a 6 or 7 out of 8 bp match with the Sp1 binding site consensus sequence, (C/T)CCGCC(C/A) (K. Jones and R. Tjian, personal communication). This partial homology raises

the possibility that these two recognition sequences share a common evolutionary origin.

Our experiences have shown that the multiplicity of promoter elements and their potential to interact can hamper the interpretation of simple promoter mutant studies. The use of synthetic oligonucleotides to verify promoter sequence functions allows a complete dissection and definitive analysis of complex promoters. The identification of promoter elements with specific functions will eventually allow the production of synthetic promoters with designed responses to various environmental or developmental signals.

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Errata

Lymphokine dependence of *in vivo* expression of MHC class II antigens by endothelium

G. Groenewegen, W. A. Buurman & C. J. van der Linden
Nature **316**, 361-363 (1985)

IN the fifth line of the fourth paragraph, the drug concentration of cyclosporin A should be 0.2 and 0.4 mg l⁻¹.

Birth of the maser and laser

Nature **316**, 307-309 (1985)

IN Professor Alfred Kastler's review of Bertolotti's book *Masers and Lasers*, two of the references are in error. The paper by Einstein, A. (1917) "Zur Quantentheorie der Strahlung" appears in *Physikalische Zeitschrift* **18**, 121-128 (1917) not *Z. Phys.*; and the paper by Boulouch, R. "Dédoublément des Franges D'Interférence en lumière naturelle" appears in *Journal de Physique Théorique et Appliquée* **2** (3rd Ser.) 316-320 (1893), not *J. Phys.*

Regulatory elements controlling chorion gene expression are conserved between flies and moths

S. A. Mitsialis & F. C. Kafatos
Nature **317**, 453-456 (1985)

THE words on the cover of the 3 October issue should have read "Moth genes into flies" and not vice versa.

Corrigendum

Amino-acid sequence of a Ca²⁺ + Mg²⁺-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence

D. H. MacLennan, C. J. Brandl, B. Korczak & N. M. Green
Nature **316**, 696-700 (1985)

ON page 697, the *M_r* of the ATPase should be corrected to 109,529; that of fragment A₂ to 22,089; fragment A₁ to 33,423; and fragment B to 54,053.

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